MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. XXXV January–February, 1943 No. 1

DICTYOPHORA DUPLICATA

Fred J. Seaver

(with frontispiece)

It has been customary in time past to publish outstanding photographs of fungi in Mycologia, and the practice should be continued when such are available. We have recently received from Maurice B. Walters what we regard as a rather striking illustration of the above named species, with the following notes:

"On Sept. 18th I found, in beech-hemlock woods near Cleveland, the largest and most perfect specimen of *Dictyophora duplicata* I have ever seen, a trifle over nine inches high, and with net unbroken. I succeeded in getting it home undamaged, and my resulting photo also came thru without a flaw. I have made a life-size enlargement and am sending it to you under separate cover. I know you sometimes make use of photos of unusual specimens for Mycologia; of course I do not know how unusual this one is, but I have not seen any published photograph of such a flawless specimen as this."

Mr. Walters is to be commended for his good fortune in finding such a magnificent specimen, and getting it home without injury; also for his ability as a photographer. We take great pleasure in reproducing this unusually fine illustration, and would be glad to have similar contributions from other mycologists.

New York Botanical Garden

[Mycologia for November–December (**34**: 601–724) was issued December 1, 1942]

AN UNDESCRIBED LAGENIDIUM PARASITIC UPON POTAMOGETON

V. M. Cutter, Jr.

(with 21 figures)

Phycomycetous parasites in the root hairs of terrestrial plants have been reported in numerous instances, but the presence of these organisms in the root hairs of aquatic plants has been largely overlooked. This paucity of records may be due to the fact that, until recently, the presence of root hairs on the great majority of aquatic plants had not been realized. Consequently, it is of interest to record the occurrence of a Phycomycete parasite infecting the root hairs of a true aquatic and causing a marked hypertrophy of these structures.

In May 1941, Dr. W. C. Muenscher of this University called the writer's attention to a collection of seedlings of *Potamogeton spirillus* Tuckerman with root hairs showing curious pyriform or spherical swellings. Many of these swollen hairs were hyaline and completely empty, but spore-like bodies which resembled *Pythium* oögonia were present in a few. There was, however, no trace of any mycelium in either the root hairs or the cells of the root. Further search revealed root hairs in which the protoplasmic contents had apparently been incorporated into an amoeboid mass lying in the swollen tip of the hair. These lobulated masses were immediately suggestive of the thalli of the Lagenidiaceae. During the course of the preliminary study, one of these amoeboid thalli produced a number of zoöspores; but it was not possible at the time to ascertain definitely the flagellation of these swarmers. A second collection of more mature seedlings taken from the same locality on July 2, 1941, showed an occasional infected root hair; but in this instance, only the amoeboid thalli were present, and zoöspore discharge was not observed. Obviously the affinities of this organism could only be determined by a careful study of its zoöspores. With this in mind, another collection was made from

the same station on May 27, 1942. In this third collection the parasite was again present, and a more representative series of stages was obtained. A final collection made July 2, 1942, showed many empty root hairs and the presence of a few vegetative and resting thalli.

HABITAT

The seedlings of *Potamogeton spirillus* Tuckerman were collected at two localities in Oquaga Lake, Broome County, New York, the first in slowly running water near the outlet and the second in a boat slip at the western end of the lake. This lake lies at an elevation of 1571 feet and, judging from the vegetation, shows an acid reaction. At the time of collection the water was in the 40–60° F. range. The plants were growing in 1–3 feet of water, and were all seedlings. Mature plants collected at the outlet showed no infection. In the heaviest infection seen, that of May 30, 1941, approximately 50 per cent of the root hairs showed hypertrophy. In the 1942 material infection was much less severe, only an occasional root hair showing the presence of the fungus. Seedlings of *Sparganium, Eriocaulon, Juncus, Eleocharis* and *Callitriche,* and young plants of *Isoetes* taken from the vicinity of the infected *Potamogeton* showed no trace of infection. A number of other species of *Potamogeton* and *Najas* seedlings from other localities were examined in the hope of finding the same fungus, but no similar infection has been noted. It is possible, of course, that this fungus may be specific on *P. spirillus.*

DEVELOPMENT OF THE ZOÖSPORANGIAL THALLUS

The first evidence of the parasite's presence in the root hair is a gradual disappearance of the protoplasmic contents and an enlargement of the tip of the hair. The thallus appears plasmodial at first; but soon a definite membrane may be distinguished, and at this time the contents of the thallus become granular and oil droplets are formed in the cytoplasm. The bright blue or purple reaction obtained when the thallus membrane is treated with chloriodide of zinc indicates that true cellulose is present in the cell wall. This suggests a close affinity with the Lagenidiaceae, in which

Couch (2) and others have demonstrated the presence of cellulose, and with the higher Phycomycetes. Considered in conjunction with the nature of the zoöspores, this character further proves the impossibility of grouping this fungus with the Chytridiales.

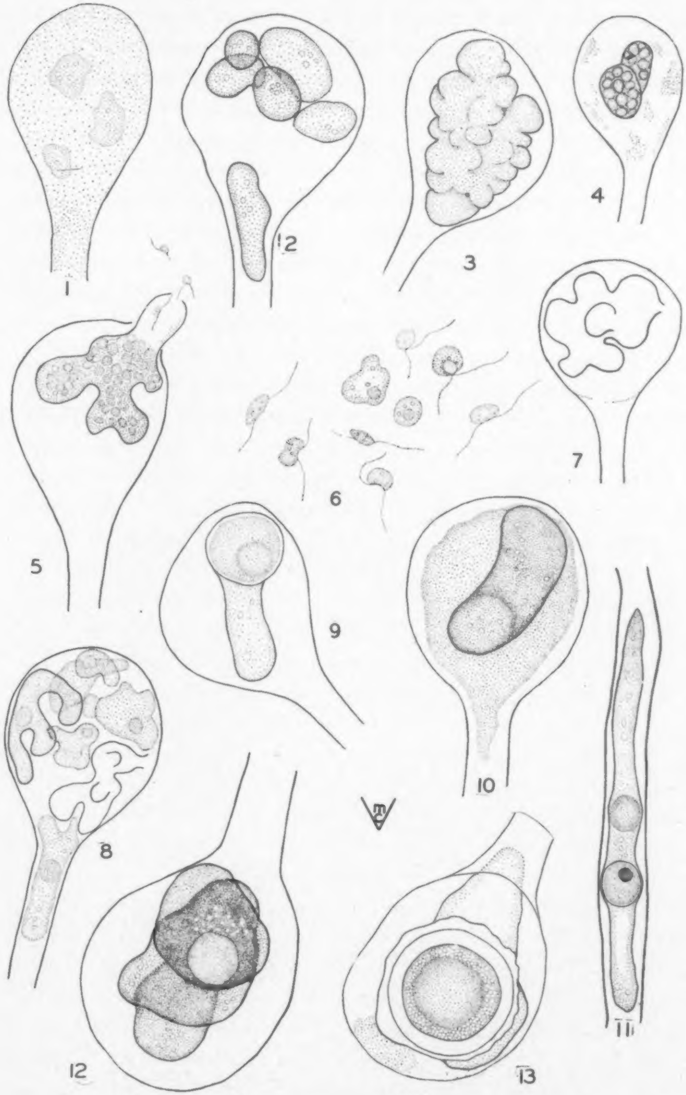
As the thallus matures, it assumes a pale golden color with a greenish gleam. The thalli are at first subspherical or elliptical, becoming much lobed and convoluted in age (FIG. 3). The spherical thalli may reach a diameter of 90 microns, while elongated individuals may attain even greater dimensions. Several root hairs were observed in which more than one thallus had developed (FIG. 2), and as might be expected, these thalli were much smaller. One such thallus only 12 microns in diameter was observed discharging zoöspores. No septa were seen even in mature thalli.

The fungus is usually confined to the terminal portion of the root hair, where it causes a very marked balloon-like swelling. In none of the material examined was there any evidence that the parasite had infected the epidermal or cortical cells of the root, although many zoöspores were observed coming to rest and encysting upon the root epidermis. This may indicate that the zoöspores are unable to penetrate mature cell walls and can gain entrance to the host only through the very delicate or perhaps injured walls of the expanding root hairs. The infected hairs are almost always shorter than the healthy ones; and growth of the hair apparently ceases after infection has occurred. This may be further evidence that infection only takes place when the root hairs are immature. Aside from this local hypertrophy, the infected seedlings appeared normal in every way and exhibited no pathologic symptoms.

ZOÖSPOROGENESIS

Very shortly after the thallus has reached its maximum development a number of cyst-like bodies measuring 6–12 microns may

FIG. 1, young thalli in root hair, × 285; 2, root hair with several young thalli, × 285; 3, mature lobulated thallus, × 285; 4, thallus before zoöspore discharge, showing pseudocysts, × 285; 5, zoöspore discharge, × 285; 6, swimming and encysted zoöspores, × 510; 7, root hair with empty thallus, × 285; 8, empty zoösporangial thallus and several young resting thalli arising from trapped zoöspores, × 285; 9–10, development of oösphere, × 330; 11, elongated resting thallus with developing oöspores, in stalk of root hair, × 330; 12, developing oösphere in lobe of thallus, × 330; 13, mature oögonium and oöspore, × 330. All figures drawn with the aid of a camera lucida.



FIGS. 1–13.

be observed within it (FIG. 4). These remain motionless for several hours and then begin to show amoeboid movements. As the cysts change shape the whole thallus, or sometimes only portions of it, commence to rock and jerk about. The nature of these cyst-like bodies is not clear, but observations at a later stage indicate that they probably represent only the zoöspore membranes, since no empty cysts remain in the thallus after the zoöspores have swarmed, as would be the case if the zoöspores went through a preliminary encystment prior to emergence. However, the outlines of the developing zoöspores in these early stages are much more apparent than is usually the case among Water Moulds; and this phenomenon deserves further study, since it may represent a rudimentary type of sporangial encystment, and thus shed light upon the development of true sporangial encystment in such genera as *Thraustotheca* and *Dictyuchus*.

The zoöspores (FIG. 6) are of the familiar biflagellate "grape-seed" type with a number of refringent granules scattered in the cytoplasm. They measure 6–11 × 3–5 microns and swim with a smooth rolling motion quite unlike the erratic darting of chytridiaceous zoöspores. They are isocont, with prominent flagella measuring up to 25 microns in length. They may remain motile for as long as 12 hours if the oxygen supply of the culture is maintained; but they usually settle down and become amoeboid within several hours, and as their amoeboid motion ceases they round up and encyst. No repeated emergencies of encysted zoöspores were observed, nor were any zoöspores seen to effect entrance to the host, although many encysted zoöspores were observed at rest on healthy root hairs. The question of how infection is accomplished must remain for the present unanswered.

In a number of cases one of the lobes of the thallus was seen to have penetrated the root hair wall just before zoöspore discharge. This portion of the thallus was not particularly differentiated into an exit tube or pore, and the zoöspores were released individually through a simple orifice or break in the projecting lobe (FIG. 5). Infrequently the thallus may produce an elongated exit tube through which the zoöspores are discharged. At the time of release no vesicle or membrane was present into which the zoöspores were discharged. The entire thallus may not be

emptied for several hours. How the thallus accomplishes the rupture of the root hair wall remains in the realm of conjecture. Evidence would favor the theory that enzymatic action dissolves the root hair wall, since the thallus rarely occupies the entire swelling and would, therefore, probably not build up sufficient pressure to cause mechanical rupture of the root hair. On the other hand, the break may well be caused by a tearing of the delicate hair wall as the root elongates, the fungus playing only a passive role in the process. In many instances, zoöspores were discharged from thalli before the root hair was ruptured, and in these cases the trapped spores swam about in the terminal swelling and finally came to rest and encysted *in situ*. This situation presented an unusual opportunity to follow the subsequent course of development of the fungus. The thalli which develop the resting spores apparently arise from these trapped zoöspores.

DEVELOPMENT OF OÖGONIA AND OÖSPORES

After several days in the encysted condition, the zoöspores begin to increase in size and young plasmodial thalli are again in evidence (FIG. 8). By the end of the fourth day after encystment, these daughter thalli are practically as large as the original thallus and differ from it only in the presence of a very large and conspicuous oil globule (FIG. 12). This globule may lie centrally in the thallus or develop in one of the lobes. As differentiation proceeds, the cytoplasm gradually accumulates around the globule soon forming a well demarcated oösphere (FIG. 9). A distinct periplasm is not evident. Soon a conspicuous hyaline to golden yellow wall up to 2.5 microns thick is formed about this oösphere, and the oöspore now lies free within the thallus. The thallus wall therefore represents an oögonium. All the protoplasmic contents of the thallus are not necessarily incorporated within this oöspore, and one or more lobes may remain undifferentiated (FIG. 13). It is problematical whether these lobes function as antheridia, but since no male organ nor any fertilization process was seen it appears likely that the development of the oöspore is apandrous. A particular effort was made to determine whether any fusions occurred between different thalli lying in the root hair

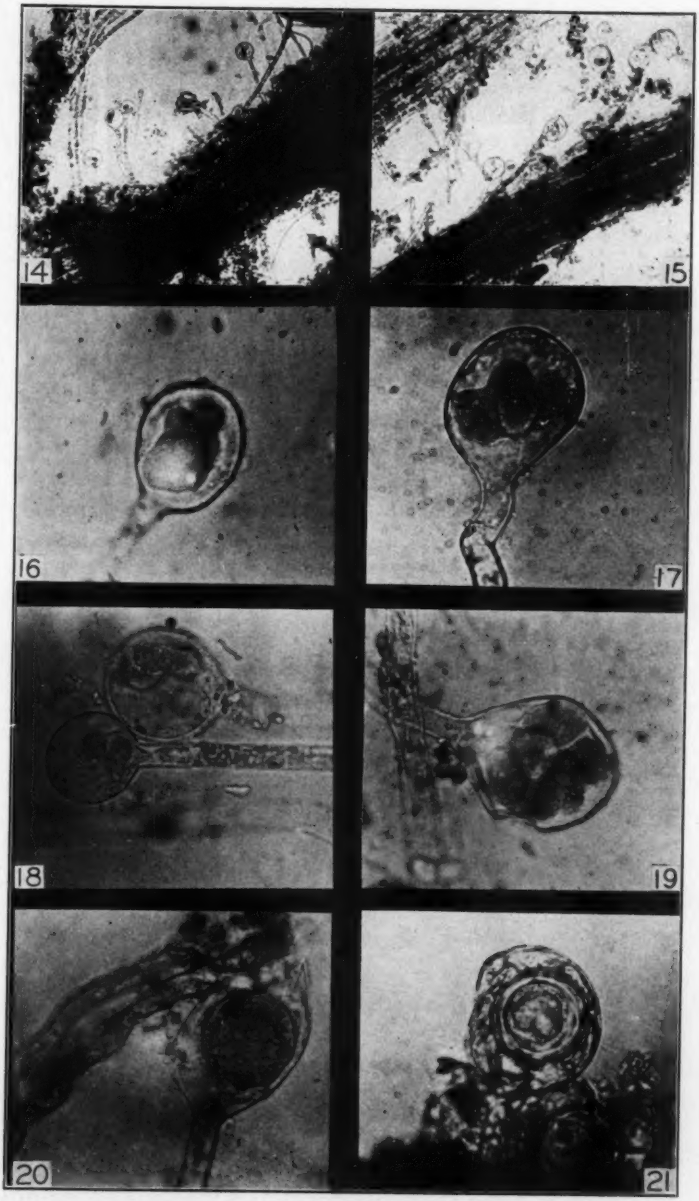
but in the many examples observed no indications of this were noted.

The oöspores range from 10 to 30 microns in diameter, and at maturity have a golden yellow color. In age the irregular contour of the oögonial wall gives them an appearance not unlike the oögonia of some species of *Aphanomyces*. It must be emphasized, however, that the oögonial wall is not decorated with definite protuberances, but only appears so, due to the uneven wrinkling of the external membrane. A few thalli with developing oöspores were noted in the stalks of the root hairs and the terminal swellings were empty (FIG. 11). These presumably arose when zoöspores discharged from a thallus lying in the terminal portion of the hair swam down the stalk and encysted there. Thalli occupying this position were usually considerably elongated, and did not cause any marked hypertrophy. The fact that in all the cases observed the oögonial thalli caused no further hypertrophy, beyond that already induced by the previous presence of zoösporangial thalli, may indicate that the resting spores are only produced upon secondary thalli which develop in previously infected root hairs where the protoplasmic contents have been largely exhausted. Further observation will be necessary to clarify this situation. The germination and subsequent fate of the oöspores has not been observed.

SYSTEMATIC POSITION

This organism possesses certain characteristics intermediate between the Olpidiopsidaceae and Lagenidiaceae of the order Lagenidiales in the classification of Sparrow (**6**). In the apparently naked, plasmodial-like appearance of the young thallus and the holocarpic nature of the mature thallus, as well as the failure to develop a vesicle into which the zoöspores are discharged, this fungus shows affinities with *Olpidiopsis* Cornu. On the other hand the large size of the zoöspores and the well developed oöspore with eccentric oil globule and smooth wall indicate a close relation-

FIGS. 14–15, infected root hairs of *Potamogeton*, × 50; 16–17, mature zoösporangial thalli, × 160; 18–19, root hairs with several young resting thalli, × 160; 20, thallus with developing oösphere, × 160; 21, mature oögonium and oöspore, × 160.



FIGS. 14–21.

ship with *Lagenidium* Schenk. The *Potamogeton* parasite differs from both these genera in its occurrence on an angiospermous host, in which it shows a similarity with *Lagena* Vanterpool & Ledingham. The importance of host relationships in determining the systematic position of parasitic members of the lower Phycomycetes has never been carefully expounded, and for this reason too much emphasis cannot be laid upon such a character in making generic distinctions. The three most important aspects in which this fungus differs from *Lagena* are: the absence of a definite neck or attachment collar on the thallus, which remains in contact with the host wall at the point where the zoöspore gained entrance to the host; the lack of a discharge vesicle; and the fact that the *Potamogeton* parasite is not dioecious. The first character is undoubtedly of great importance, while the latter two may be of only incidental interest from a systematic point of view. Superficially this fungus resembles *Myzocytium zoopthorum* Sparrow (**4**) in the absence of a discharge vesicle, but differs from typical members of *Myzocytium* Schenk in that the thallus is never segmented or link-like.

Only two characters controvert placing this organism in the genus *Lagenidium*. These are the lack of a sporangial vesicle about the zoöspores at the time of their discharge, and the apparent lack of a well defined antheridial branch on the sexual thallus. The nonseptate character of the thallus might also be raised as an objection to placing it in this genus. Although it may be emphatically stated that in the present material no sporangial vesicle was present and the zoöspores were delimited within the thallus and discharged individually, it is equally probable that under different environmental conditions a vesicle may be formed. The accounts of several workers notably Atkinson (**1**), Scherffel (**3**), and Sparrow (**5**) indicate that in *Lagenidium* a vesicle is not invariably present, and at best its presence may be only momentary. In regard to the absence of an antheridial branch on the sexual thalli two explanations present themselves. Either this structure is transitory and collapses immediately after fertilization and has thus been overlooked, or conjugation may be lateral as in the genus *Myzocytium* with one of the undifferentiated thallus lobes functioning as the male gametangium. In addition to these factors the possi-

bility that the fungus is naturally apandrous must be considered. This latter explanation appears more valid since there is little difficulty in clearly observing the thallus as it lies in the hyaline root hair and the presence of an antheridial branch could be easily determined were it present. The non-septate thallus makes the possibility of lateral conjugation unlikely since it is difficult to conceive of a unicellular organism physiologically differentiated into oppositely sexed portions. This lack of septation while unusual in *Lagenidium* has nevertheless been reported in a number of species. It probably represents a primitive condition. The occurrence of apandrous strains is not unusual among the Phycomycetes, and should not stand in the way of assigning an organism to a genus in which true sexuality is the general rule.

From previously described species of *Lagenidium* this fungus may be separated by its large size, parthenogenetically developed oöspores, and occurrence on an angiospermous host as well as by the curious pseudoencystment of the zoöspores just before their release from the thallus. For these reasons it seems best to consider this as a somewhat anomalous species of *Lagenidium*. It is therefore named in honor of Dr. W. C. Muenscher who first collected it, and whose aid in making subsequent collections has been invaluable in this study.

Lagenidium Muenscheri sp. nov.

Thallo intramatricalis, monocentrico, holocarpico, primum nudiusculo, maturitate distincto pariete cellulosam habiente disiuncto, sub-globoso, elliptico, cylindrico, aut lobulo et convoluto, non diviso, 12–20 μ in diam., zoosporis per oro in thalli mure liberatis. Zoosporis hyalinis, minutissime refringentis granulatis, reniformi, lateraliter biflagellatis, isoconti, 6–11 μ × 3–5 μ, maturis emergentibus subitoque enantibus, aequaliter haud emicatim movent, tandem amoeboideis et encystantes, formatis novo thallo directo germinatis. Thallo perdurans apandro, in oogonium transformatur, 40–110 μ in diam. plerumque lobulo. Oosporae sphaericae, parthenogeneticis, flaventes, 18–30 μ in diam. cum eccentrico globulo, murus 1–2.5 μ crassus, germinatio non observata.

Thallus intramatrical, monocentric, holocarpic, appearing naked when young, becoming invested with a cellulose wall at maturity, subspherical, elliptical, elongated or lobed and convoluted, non septate, 12–90 μ in diam., liberating zoöspores through an orifice in the thallus wall. Zoöspores hyaline with numerous minute refringent granules, reniform, laterally biflagellate, isocont, 6–11 μ

$\times$ 3–5 μ, emerging fully formed and swimming away directly (with even non-darting motion) finally becoming amoeboid and encysting, upon germination forming a new thallus directly. Resting thallus apandrous, transformed into an oögonium, 40–110 μ in diam., usually lobed. Oöspores spherical, parthenogenetic, golden 18–30 μ in diam. with eccentric oil globule, oöspore wall smooth, 1–2.5 μ thick. Oöspore germination not observed.

Parasitic upon and causing a hypertrophy of the root hairs of *Potamogeton spirillus* Tuckerman. Type locality: Oquaga Lake, Broome County, New York, May–July 1941–1942. Coll. W. C. Muenscher. Type specimen No. *1001* in herbarium of author at Cornell University.

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VARIATIONS IN SPORULATION OF DIFFERENT ISOLATES OF COLLETOTRICHUM DESTRUCTIVUM[1]

S. J. P. Chilton[2]

(with 2 figures)

Single spore cultures of *Colletotrichum destructivum* O'Gara were found to lose their ability to produce conidia in any quantity when kept in culture. As this seems to be a common phenomenon with several species of *Colletotrichum* and the closely related genus *Gloeosporium*, a study was made of *C. destructivum* to determine, if possible, the nature of this loss of sporulation. A brief summary has been published previously of the results given in this paper.[3]

MATERIALS AND METHODS

Fourteen single spore cultures from four hosts were used in the studies (Table 1). All single spore cultures were made with a glass needle and micromanipulator. Unless stated, cultures were compared in triplicate or quadruplicate plates of potato dextrose agar.

Special techniques are given in the experiments in which they were used.

EXPERIMENTAL RESULTS

Description of the original cultures. The 14 cultures used in the studies differed somewhat in cultural characters. Most of

[1] Contribution No. 39 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in coöperation with the Northeastern States.

[2] Formerly Agent, U. S. Regional Pasture Research Laboratory; now Assistant Professor, Department of Botany, Bacteriology, and Plant Pathology, Louisiana State University.

[3] Chilton, S. J. P. Loss of sporulation in *Colletotrichum destructivum* (Abstract). Phytopathology **31**: 5-6. 1941.

them were nearly black due to the occurrence of small masses of black mycelium scattered in and on the agar. Conidia were plentiful. One culture was lighter in color and produced a pink mass of spores over the center of the colony. The surrounding lighter area was sprinkled with small black masses of mycelium.

Loss of sporulation. When cultures were kept for approximately six weeks or more in tubes of agar, a patch of white, fluffy mycelium would usually appear and spread gradually over the surface of the original growth. If transfers were made to fresh

TABLE 1

SOURCE OF CULTURES OF *Colletotrichum destructivum*

Culture number	Date isolated	Host	Locality
5-1	7/15/38	*Trifolium repens*	State College, Pa.
6-1	7/15/38	*Trifolium repens*	State College, Pa.
14-1	7/18/38	*Trifolium repens*	State College, Pa.
18-1	7/18/38	*Trifolium repens*	State College, Pa.
24-1	7/18/38	*Trifolium pratense*	State College, Pa.
32-1	7/21/38	*Trifolium pratense*	State College, Pa.
150-1	7/29/38	*Trifolium hybridum*	State College, Pa.
224-1	8/13/38	*Trifolium repens*	State College, Pa.
225-1	8/13/38	*Trifolium repens* var. *Ladino*	State College, Pa.
227-2	8/13/38	*Trifolium repens* var. *Ladino*	State College, Pa.
260-1	8/16/38	*Trifolium repens* var. *Ladino*	West Newton, Pa.
329-1	8/21/38	*Trifolium repens*	West Newton, Pa.
332-1	8/21/38	*Trifolium repens*	West Newton, Pa.
338-1	8/21/38	*Trifolium hybridum*	West Newton, Pa.

tubes of agar, the resulting growth seemed to be: (*a*) a mixture of the original type and the white, fluffy mycelium; (*b*) only the second type; or (*c*) a mixture of several types. If transfers were made to plates of agar, the resultant growth would often appear to have sectored (FIG. 1, bottom).

Single spore cultures were made from the tubes in which the fluffy mycelial patches occurred and also from the sectors appearing in petri plates. These cultures when compared differed in many characters. Some produced a few spores and were characterized by a white, fluffy mycelium. Others produced many more conidia than the original type from which they arose, the surface being covered by a pink mass of conidia. Table 2 gives the number of variant types obtained from the 14 cultures. It may be seen that as many as 15 distinct types were secured from

FIG. 1. Top, original culture of *Colletotrichum destructivum;* middle, nine variant types from original culture; bottom, culture showing sectors.

one original culture. All the cultures produced one or more poorly sporulating white mycelial variants. A total of 57 distinct cultural types were obtained in all, these differing in rate of growth, color, zonation, the presence or absence of setae, and amount of conidia produced. Figure 1 shows the original culture 14–1 and 9 variant types secured from it.

If enough single spore isolates were made from a culture beginning to change from the sporulating to the poorly sporulating type, the original and a poorly sporulating variant type were always obtained. As many as five variant types were found among the single spore isolates made at one time from such a culture, these differing in color, amount of conidia, and other characters.

TABLE 2

NUMBER OF VARIANT TYPES ARISING FROM 14 SINGLE SPORE CULTURES OF *Colletotrichum destructivum*

Culture	Number variant types	Culture	Number variant types
5-1	4	226-2	2
6-1	5	247-2	6
14-1	15	259-1	3
24-1	10	260-1	4
32-1	11	329-1	10
150-1	10	332-1	4
224-1	10	338-1	2

The same poorly sporulating white mycelial type arose in each of three successive single spore generations from the original type. Once the original cultural type produced two variants, one the poorly sporulating type and the other a culture which sporulated much more profusely than the original culture from which it was obtained. A single spore culture was made from it which later produced a variant indistinguishable on potato dextrose agar from the poorly sporulating variant obtained from the original culture.

From 2 to 5 successive single spore cultures were made from many of the variants. In all cases these variants retained their identity although the successive single spore cultures from them often produced other types.

In order to determine whether fresh spore isolates could produce similar variants, 1,200 single spores were isolated from nine

cultures, care being taken that the cultures had not been on agar longer than 20 days from single spore to single spore. Only two variants were obtained.

Nuclear condition of conidia. Conidia were fixed and stained by the iron-alum hematoxylon technique to determine the number of nuclei present. Figure 2 shows that they were predominantly uninucleate. Of 11,330 spores from eight cultures, 35 contained two nuclei and the remainder were uninucleate.

The effect of successive single sporing. Single spore cultures were made and placed in tubes or petri plates. When growth covered the surface of the agar the process was repeated. This was

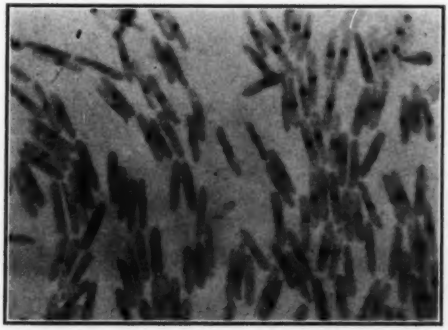


FIG. 2. Spores of *Colletotrichum destructivum* showing uni-nucleate condition.

done for 10 successive generations with one culture, 8 with another, and 4 with two more. The cultures were the same with respect to sporulation and other cultural characters at the end of the experiment.

To calculate the dilution of any material present in the original spore in successive single spore cultures, the following was done: The diameter and thickness of the mycelial mat on the agar surface was measured and its volume calculated. One-tenth of this mass was arbitrarily considered to be fungus tissue. The ratio between this volume and that of a single spore was calculated. After four successive single spore generations the calculated dilu-

tion was 7.2×10^{30}, after eight, 6.58×10^{67}, and after 10 single spore generations it was 1.8×10^{83}.

Effect of sterile host tissue on poorly sporulating variants. It has been reported that cultures which were losing their ability to sporulate could be rejuvenated by transferring to sterilized host tissue and maintaining them on this medium for several transfers. To test this, petioles and leaflets of *Trifolium repens* were sterilized for 20 minutes at 15 pounds pressure. Tubes were inoculated with each of five poorly sporulating variants and other tubes with the original cultures from which they arose. Periodic transfers were made every 2 to 4 weeks to new host material and at the same time to agar plates to determine if any change occurred. At the end of five successive transfers no difference was found in the 10 cultures used.

In another experiment, cultures which were losing their ability to sporulate were transferred to sterilized host tissue and single spore isolations made. The original sporulating type was obtained.

Effect of the living host on poorly sporulating variants. Repeated passage through the living host has been reported to cause cultures to regain their ability to sporulate. An experiment was made to determine if this occurred with *Colletotrichum destructivum.* Cultures were grown on sterile red clover seedlings in flasks obtained in the following manner: Seed was used which had been tested for the presence of *C. destructivum* by surface sterilizing in 95 per cent alcohol for one minute, a 1–1,000 aqueous solution of mercuric chloride for seven minutes, and a saturated solution of calcium hypochlorite until plated on potato dextrose agar. As none of the 3,000 seed tested in this manner produced the fungus, macroscopically sound seed were selected, surface sterilized as above, and placed in 250 cc. Erlenmeyer flasks containing 50 cc. of potato dextrose agar. After the seed germinated and produced seedlings about three inches in height, all contaminated flasks were discarded. Three single spore cultures of the poorly sporulating type were inoculated on the seedlings. When infection occurred they were transferred to new flasks of seedlings, and, in turn, to other seedlings. On re-isolation to plates the cultures were the same poorly sporulating types.

The occurrence of variants on the living host. In another experiment single spore isolates from two of the original type cultures were made on small pellets of agar 5 mm. in diameter. As soon as the spore produced sufficient mycelium to cover the pellet, half was taken and put in a tube of agar to make sure of the cultural type and the other half was placed on sterile red clover seedlings in a flask. When infection occurred single spores were isolated from those produced on the infected tissue. In all, 986 cultures were isolated and compared in test tubes. One culture, 14–1, produced two variants among 414 isolates. One of these variants was the poorly sporulating white mycelial type and the other differed in other cultural characters.

DISCUSSION

The results obtained indicate that the cause of the loss of sporulation in *Colletotrichum destructivum* in cultures is the occurrence of poorly sporulating variants that replace the original type. These variants were not due to a heterocaryotic condition of the mycelium, and all attempts to rejuvenate them were fruitless. The evidence indicates they are genetic entities differing from the cultures from which they arose. It seems, therefore, that they arise as mutants. The possibility exists that the nuclei in the mycelium are heterozygous diploids which could fuse and segregate in the mycelium, giving rise to variant types.

SUMMARY

1. Studies were made of 14 cultures of *Colletotrichum destructivum* with respect to the loss of sporulation.

2. Variant types were isolated from sectors and patches in old cultures which differed from the original in various cultural characters. Some produced very few spores while others produced many more spores than the original cultures from which they came.

3. The original cultures retained their ability to sporulate through 10 successive single spore generations. Transfers to sterile host tissue and to the living host did not cause poorly sporulating cultures to regain their ability to sporulate heavily.

4. Two variants were obtained from lesions on red clover seedlings produced under sterile conditions.

5. Conidia were predominantly uni-nucleate, a few being found which were bi-nucleate.

6. It is concluded that the loss of sporulation in cultures was due to the occurrence of poorly sporulating types genetically different from the original type which they replaced.

STUDIES IN THE GASTEROMYCETES VII

THE GENUS SCHIZOSTOMA

W. H. Long and David J. Stouffer

(WITH 8 FIGURES)

This paper discusses the taxonomic position of *Schizostoma,* gives an emended description of genus and species and records new data on their distribution.

The genus *Schizostoma* was proposed by Ehrenberg in manuscript for a plant collected in Equatorial Africa and named by him *Schizostoma laceratum.* This genus was not recognized at first and the plant was published by Fries (**1829**) as *Tylostoma laceratum* (Ehrenb.); then Leveille (**1846**) restored the Ehrenberg genus and listed the plant as *Schizostoma lacerum,* but he also included in this genus all species of *Tylostoma* with irregular mouths. Lloyd (**1904**) in his discussion of *Schizostoma* was the first to clearly set forth the true position of this genus and to give an adequate description of its characters. In most of the literature prior to Lloyd's publication it was classed as a *Tylostoma.*

The sub-family, Tylostomoideae, consists of three closely related genera, *Tylostoma, Queletia* and *Schizostoma.* All have sporophores with a specialized fibrous, hollow stem which in the unexpanded stage is a short plug included in the base of the spore sac. The stem emerges from the sporocarp on elongation but remains attached to its base in a socket. This socketed stem is a constant character of all three genera; in some species the apex of the stem is very loosely attached and is easily separated from the spore sac as in *Queletia* and certain species of *Tylostoma,* while in *Schizostoma* the stem is firmly attached in the socket to the endoperidium. The main difference between the three genera is their method of dehiscence.

KEY TO THE SUB-FAMILY TYLOSTOMOIDEAE

Sporocarp with a definite apical stoma .... *Tylostoma.*
Sporocarp dehiscing irregularly from the top as in *Calvatia* ...... *Queletia.*
Sporocarp dehiscing by irregular stellate rays along
definite sutures .... *Schizostoma.*

Sultan Ahmad (**1941**) in a recent article on the Gasteromycetes of the Panjab Plains, transferred *Schizostoma* to the genus *Queletia.* He claims that the dehiscence and glebal characters are the same and that the two genera differ only in the smaller size of the sporophore and the non-scaly striate stipe of *Schizostoma,* hence should be combined under one genus.

We have carefully examined authentic specimens of *Queletia* from Trexlertown, Penna., U. S. A. (FIG. 7) and from Seuz Sevres, France (FIG. 8) and find that the *dehiscence* of *Queletia* is a crumbling of the top portion of the peridium exactly as in the genus *Calvatia,* with the fragments falling away in succession from the top of the spore sac downward, in fact, *Queletia* is a *Calvatia* with a stalk as far as its dehiscence is concerned. Now in *Schizostoma* we have an entirely different structure and method of dehiscence, the peridium ruptures along definite sutures (FIG. 1) thus forming valves which expand into star-like rays (FIG. 1, 2, 3), these remain intact on the old plants long after the gleba has disappeared (FIG. 4–6). A comparison of figures 7 and 8 of *Queletia* with figures 1–6 of *Schizostoma* gives a clear idea of the existing differences in manner of dehiscence between the two genera. The capillitia and spores are also different as a study of the gleba of each genera shows. In view of the above data it is evident that the two genera are so different that they cannot be combined under one genus as proposed by Ahmad.

SCHIZOSTOMA Ehrenb. in Lév. Ann. Sci. Nat. III. **5**: 165. 1846.

Sporophore hypogeous in early stages, erumpent and stipitate at maturity; *peridium* of two layers, an exoperidium and an endoperidium; *exoperidium* a sandy coat; *endoperidium* membranous; *dehiscence* by definite sutures in the endoperidium, which rupture into irregular stellate rays; *sterile base* none; *stipe* central, hollow; *gleba* consisting of capillitium and spores; *capillitium* deeply colored, aseptate; *spores* continuous, smooth.

HABITAT: growing in arid or semi-arid regions.

TYPE SPECIES: *Schizostoma laceratum* Ehrenberg.

DISTRIBUTION: Africa; Asia; North America.

SCHIZOSTOMA LACERATUM Ehrenb. Ann. Sci. Nat. III. **5**: 165–166. 1846.

1829—*Tylostoma laceratum* (Ehrenb.) Fries, Syst. Myc. **3**: 44.

1846—*Schizostoma lacerum* Lév. Ann. Sci. Nat. III. **5**: 165–166.

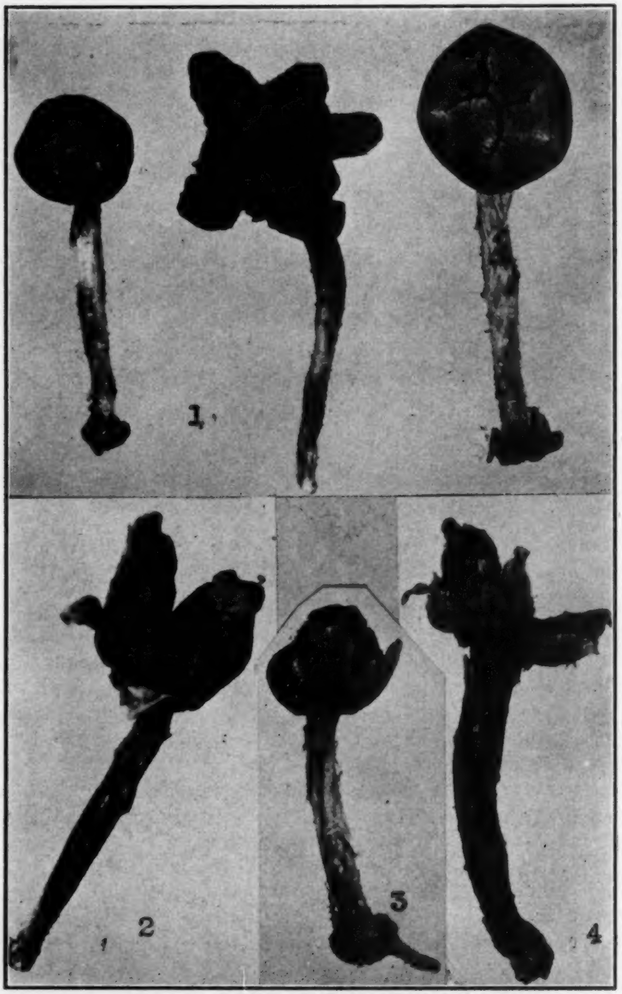
1892—*Tylostoma Schweinfurthii* Bres. in P. Henn. Bot. Jahrb. **14**: 359.

1939—*Tylostoma laceratum* var. *nigrum* S. Ahmad, Jour. Ind. Bot. Soc. **18**: 56–57.

1941—*Queletia laceratum* (Ehrenb.) S. Ahmad, Jour. Ind. Bot. Soc. **20**: 135–136.

Sporophore 1½–10 cm. tall, originating 1–8 cm. below the surface of the soil. *Sporocarp* globose to depressed-globose, 1–3 cm. across by 5–20 mm. high, showing a small knob-like, very short bulge in center of glebal cavity where the stem has pushed up bottom of the spore sac. *Exoperidium*, a sandy coat completely deciduous at maturity. *Endoperidium* smooth, membranous, outer surface wood brown (Ridgeway), showing radiating cartridge buff, definite sutures or lines of cleavage (FIG. 1); *Dehiscing* by irregular stellate rays (FIG. 1–6) along the sutures; *rays* or *valves* tough, usually remaining intact long after the gleba has disappeared (FIG. 4–6) then 1–5 cm. across from tip to tip of the expanded rays which have inner surface cartridge buff after losing the gleba. *Collar* entire, very short, sharp-edged and distant from stem. *Stipe* inserted in a shallow socket in base of sporocarp, very firmly attached, 1–8 cm. tall by 3–9 mm. thick, usually equal but often tapering toward base, terete or sometimes flattened and twisted, striate to sulcate, smooth or rarely with loose thin scales, white without and within, cortex often turning brown on weathering, firm, base usually with a volva-like structure and with a strong root or radicating rhizomorph (FIG. 3). *Gleba* chestnut color, usually very coherent, soon dissipated under weathering (FIG. 2, 4, 6); *capillitium* long, tortuose, much interwoven, chestnut brown, aseptate, 7–10 microns in diameter, sparingly branched, branches short, often tapering toward ends and thinner than main branches. *Spores* subglobose to broadly oval, 4.5–5.6 microns; *epispore* chestnut color, smooth.

HABITAT: Solitary or gregarious in unshaded areas in sandy or gravelly soil.



FIGS. 1–4, *Schizostoma laceratum*, × 1.

TYPE LOCALITY: Equatorial Africa, Nubia.

Africa:

Equatorial Africa. Nubia, *several specimens* in Berlin Mus. Type of *Schizostoma laceratum*, also in Mus. de Paris:

Schweinfurth, G., several specimens in Berlin Mus. under name of *Tylostoma Schweinfurthii: Schweinfurth, G. comm P. Hennings, 1 specimen* in Lloyd Myc. Coll. no. *25531* under name *Schizostoma laceratum*. Obock, 1892(?) *1 specimen* in Patouillard Herbarium at Farlow Herb. under name *Tylostoma Schweinfurthii.* Oasis of Sahara, Adrar, Mission Gautier, July 6, 1909. *6 specimens* in Patouillard herbarium at Farlow Herb. under name *Schizostoma laceratum.*

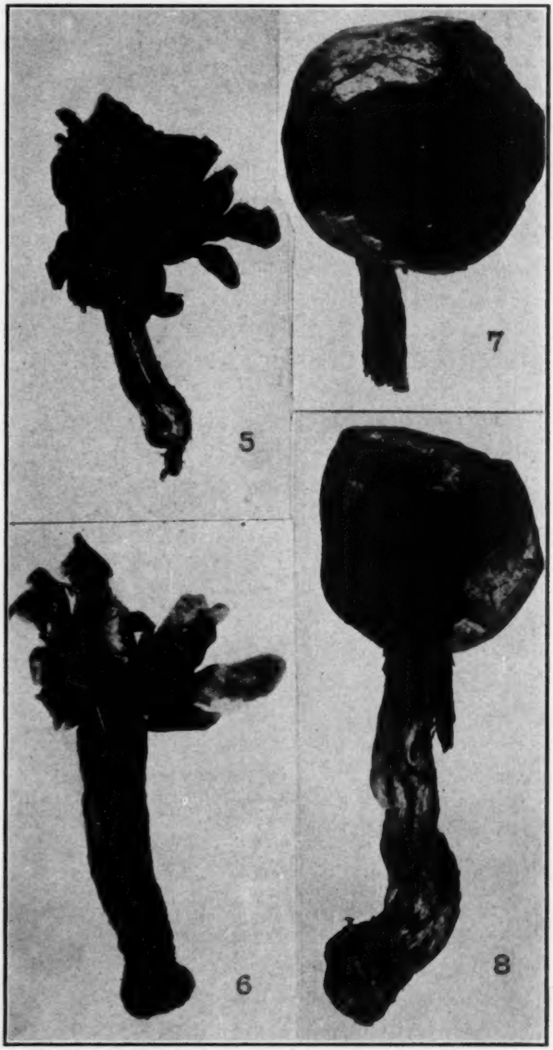
Asia:

India. Panjab Plains, Sargodha District, Sangla Hill, Jhang, Rohtak, *Sultan Ahmad, Many specimens* in Herb. Ahmad and in Herb. Crypt. Ind. Orient. of the Imperial Agric. Res. Institute, New Delhi, under name of *Tylostoma laceratum, Tylostoma laceratum* var. *nigrum*, and *Queletia laceratum; 9 plants* in herbarium of the University of North Carolina, *Sultan Ahmad* (no. 5), under name *Schizostoma laceratum.*

North America:

Arizona. Pima County, 8 miles from Tucson road to Sabino Canyon, elevation 2400 feet, *W. H. Long and Victor O. Sandberg,* Nov. 11, 1936—*1 plant* no. *7723: W. H. Long,* June 4, 1938—*20 plants* no. *8242;* Nov. 10, 1938—*17 plants* no. *8241;* Sept. 28–29, 1939—*278 plants* no. *8395: W. H. Long and David J. Stouffer,* Sept. 10, 1941—*2 plants* no. *9626.* Totalling 318 plants from Arizona.

New Mexico. Bernalillo County, 10 miles south of Albuquerque, elevation 4950, *W. H. Long,* August 20, 1941—*1 plant* no. *9477: W. H. Long and David J. Stouffer,* Dec. 6, 1941—*1 plant* no. *9920.* Dona Ana County, Jornado Experimental Range, elevation 4150 feet, *W. H. Long and David J. Stouffer,* Sept. 7, 1941—*7 plants* no. *9588;* Sept. 8, 1941—*36 plants* no. *9603, 30 plants* no. *9609* and *21 plants no. 9612: Kenneth A. Valentine,* Sept. 24, 1941—*2 plants* no. *9836.* Luna County, 10 miles west of Deming on Highway 70, elevation 4300 feet, *W. H. Long and David J. Stouffer,* Sept. 13, 1941—*1 plant* no. *9655:* making a total of 99 plants from New Mex.



FIGS. 5–6, *Schizostoma laceratum*, × 1; 7–8, *Queletia mirabilis*, × 1.

Mexico. Lower California, San Nicolas Bay, *Ivan M. Johnston* (no. *117*), May 17, 1921—*1 specimen* in Lloyd Myc. Coll. no. *25543*, under name *Schizostoma laceratum*.

Illustrations: Bot. Jahrb. **14**: *pl. 6, f. 5.* Lloyd Myc. Writ. **1**: *pl. 20, f. 1–9;* **7**: *pl. 227, f. 2324.* Jour. Ind. Bot. Soc. **18**: *pl. 2, f. 5; pl. 3, f. 1, 9.*

Schizostoma Mundkuri (S. Ahmad) comb. nov.

1941. *Queletia Mundkuri* S. Ahmad, Jour. Ind. Bot. Soc. **20**: 136–137.

Sporophore 6–18 cm. tall, originating 4–14 cm. below the surface of soil. *Sporocarp* irregular globose to depressed-globose, sometimes broader at apex, 3–5 cm. broad by 1–3 cm. high, showing a knob-like bulge in base of glebal cavity just above the stem apex. *Exoperidium,* a sandy coat completely deciduous on emergence from soil. *Endoperidium* smooth, membranous, light ochraceous buff becoming wood brown in age (Ridgway); *dehiscence* by large very irregular stellate rays along definite sutures which do not extend to bottom of spore sac; *rays* persisting long after dehiscence, rather lax and flexible under weathering. *Collar* prominent formed by the prolongation of the endoperidium, closely appressed to stem, usually very long, often extending down around top of stipe for 15 mm. and terminating in a fimbriate margin. *Stipe* inserted in a shallow socket, firmly attached to base of spore sac, 4–15 cm. tall by 1–2 cm. thick, hollow, white when fresh but part above surface of ground becoming light ochraceous buff to wood brown with age, tapering to base or sub-equal, often enlarged at apex, terete, striate to sulcate, scaly; scales thin, fibrillose, ribbon-like, fragile, resembling somewhat the scales of a *Battarrea; volva* present in specimens at hand, double, outer layer membranous with white context, covered externally with closely adhering grains of sand, inner layer of volva a mass of ribbon-like pieces with lacerate tips which apparently are fragments of the stem scales, these segments are short next to outer volva but increase in length inwardly until those next to stem may be 3 cm. long, similar in this respect to the volvas of *Battarrea digueti;* base of stem usually rooting (according to Ahmad). *Gleba* chestnut color, upper ⅔ soon dissipated under weathering, but basal portion very persistent in the lower shallow cup-like bottom of the spore sac; *capillitium* tortuose, usually long and intertwined with shorter threads, coloured to pale yellow, aseptate, very variable in thickness, 3–12.6 microns in diameter, ends closed and rounded,

sparingly branched, branches short, walls thin easily collapsing. *Spores* broadly oval to subglobose, 4.6 to 5.6 microns; *episporc* smooth, chestnut color.

HABITAT: Solitary in sandy soil. Very common in sandy wastes.

TYPE LOCALITY: India, Jhang.

DISTRIBUTION:

Asia:

India. Panjab Plains, Sangla Hill; Jhang. Rohtak; *Sultan Ahmad,* August 1939; *many specimens* in Herb. Ahmad at Rohtak and in Herb. Crypt. Ind. Orient. of Imp. Agric. Res. Inst. New Delhi. Type of *Queletia mundkuri* S. Ahmad: Rohtak, Panjab, *S. Ahmad* May 15, 1940, *4 specimens* (Ahmad no. *298*) in Long Herb. (*10262*) under name of *Queletia mundkuri.*

The above description of *Schizostoma Mundkuri* was made from material kindly sent us by Prof. Ahmad and agrees in the main with his original description of this species, but includes several important characters not mentioned by him. All of the plants in our collection from Ahmad show a definite volva, while one specimen has a large rooting rhizomorph and another has several short root-stubs on base of its volva.

Some might call this species a giant form of *Schizostoma laceratum* but we believe it merits specific rank hence it is retained as a distinct species.

American plants.

The specimens examined from the United States and Mexico all belong to the same species, *Schizostoma laceratum.*

This species is erratic in its appearance, some years being very abundant over certain areas while rare at other times, as evidenced by the collections (FIG. 2, 4, 5, 6) from near Sabino Canyon in Arizona where only 1 plant was found in 1936, 57 in 1938, 278 in 1939 and only 2 plants in 1941, yet all the collections were made on the same area (about 10 acres in size) for each of these years. This area is in a Mesquite-Catclaw flat (*Prosopis-Acacia*) with a heavy clay-sandy soil intermixed with gravel and having a

limestone subsoil. The plants on the area usually were growing around the margins of old abandoned rodent mounds.

On the Jornado Experimental Range in New Mexico, *Schizostoma laceratum*, grew in deep sand having a clay subsoil, on naked areas between the Mesquite-sandhill dunes. Many of these plants when collected September 7–8, 1941 were just emerging from the ground (FIG. 1, 3), in some instances their presence was only indicated by a slight elevation of the sand over the emerging plants; even when fully elongated many of them showed only the sporocarp above the soil, while in some cases not even all of the sporocarp became exposed.

The material collected in Mexico consisted of only 1 plant and was typical of *Schizostoma laceratum*.

Asiatic specimens.

This genus is represented in Asia by 2 species—*Schizostoma laceratum* and *S. mundkuri.* We have seen and studied collections of both of these species from India.

The material sent by Ahmad to the University of North Carolina was probably the basis of his description of *Tylostoma laceratum* in his paper (**1939**) on the Gasteromyceteae of the Punjab Plains. The plants in this collection varied in size from 2 to 6 cm. tall with sporocarps from 1 to 3 cm. broad; only one plant had any gleba remaining in the spore sac, this easy loss of the gleba is characteristic of this species. These Indian specimens corresponded in every detail to our American plants of *Schizostoma laceratum.*

Ahmad's variety—*Tylostoma laceratum* var. *nigrum*—was not in the collections from India sent to the University of North Carolina by Ahmad. According to the description, this variety differs from the ordinary species only in its small size and the darker color of the gleba. We do not believe such minor and variable characters are sufficient to warrant a variety hence it is listed as a synonym of *Schizostoma laceratum.* Recently we received direct from Prof. Ahmad some fine specimens of his new species—*Queletia Mundkuri.* The results of our study of this material have been incorporated in this paper.

The African collections.

We have examined 2 collections of *Schizostoma laceratum* from Africa. One lot of 6 plants came from an oasis in the Sahara Desert and the other consisting of one plant from Obock.

The Obock plant was 5 cm. tall with a subglobose sporocarp 3 cm. across showing the characteristic knob-like projection in its glebal cavity, exoperidium gone, endoperidium smooth, membranous, wood brown with radiating sutures which have opened into irregular stellate rays 3 cm. long, collar entire, sharp-edged; stipe firmly attached to spore sac, 3 cm. tall, tapering to base, striate, terete with a brownish cortex; gleba walnut brown to chestnut brown. The six plants of the Sahara Desert Collection from Adrar ranged in size from 5 to 6 cm. tall with stems 4–6 mm. thick, usually equal, terete, some smooth, some striate with a whitish to brownish cortex.

Schizostoma laceratum from the three continents, Africa, America and Asia has characters so similar that all plainly belong to one species, only a few minor differences, mainly in size of individual plants are evident in any of these widely separated collections, otherwise their characters are practically identical.

GENERAL REMARKS

The habitat requirements of *Schizostoma* vary greatly as to soil and temperature. In Africa, the original *S. laceratum* came from the vast hot gravel lands of Nubia, while *Tylostoma Schweinfurthii*, a synonym, was found in a similar area in Africa. In Asia according to Ahmad (**1939**) the plants were collected in the hot sandy wastes of the Punjab Plains of India. The American plants grew in a variety of soils: the Arizona specimens were in a sand-clay-gravel soil with a limestone subsoil. The New Mexico plants grew in 2 types of soil, a sandy soil with a clay subsoil in Mesquite-sandhill areas, and on sand-gravel ridges in the foot hills of the Manzano Mountains. The temperatures range from very hot in the sub-tropical zone to much colder in the temperate region where the winters may have temperatures 4–6 degrees below zero Fahr. and snows 2–3 feet deep.

The genus, *Schizostoma,* is unique in its well marked cleavage sutures of the endoperidium before dehiscence, not even in the genus *Geaster* are such sutures evident externally. Ahmad (**1939**) was the first to report the presence of these sutures. He wrote as follows, "The writer finds that the peridium ruptures along definite lines which are marked in the unopened specimens by a lighter color, and thus forming definite valves which remain intact even when the gleba is dispersed. In some specimens the endoperidium opens along the lines mentioned above even when the top portion has not broken at all."

The stipes in the various collections from the three continents show a white stem cortex when emerging from the soil, but may change to various shades of brown with age and weathering. The interior context of the stems is white in all plants and remains so even in age and under severe weathering.

This genus is probably more widely distributed in the hot, semiarid regions of the world than the above records show.

Prior to this paper only one specimen of *Schizostoma* was reported from America, a solitary plant found in Mexico and described and illustrated by Lloyd (**1922**).

ACKNOWLEDGMENTS

We wish to make grateful acknowledgments to Mr. John A. Stevenson for loan of material and many helpful suggestions on the Bibliography; to Dr. David H. Linder for loan of material and valuable suggestions on nomenclature and bibliography; to Dr. John N. Couch and Mrs. Alma H. Beers of the University of North Carolina for loan of material; to Prof. Sultan Ahmad M.Sc. of Government College, Rohtak, India for valuable material.

ALBUQUERQUE, NEW MEXICO
AND
CORONA, NEW MEXICO

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MOISTURE-RELATION AS A DETERMINANT FACTOR IN THE TRANSFORMATION OF THE BASIDIA OF CERTAIN POLYPORACEAE

S. R. Bose

(with 8 figures)

In (**1935**) I showed that in *Ganoderma lucidum* and some other Polyporaceae (*P. ochroleucus, P. calcuttensis* and *Trametes lactinea*), the basidia are succeeded at the end of the rainy season by hyphal elongations which grow out from the hymenium and develop terminal spores (*i.e.* secondary basidiospores). I then expressed the hope that in course of time many more species of Polyporaceae would be found to exhibit the same mode of reproduction by secondary spores without basidia after the rains or during intervals between two showers in the rainy season. Since then, I have extended my observations to other species of Polyporaceae (*Polyporus zonalis, P. ostreiformis, P. rubidus, P. gilvus, P. anebus, Polystictus sanguineus, P. versicolor, P. hirsutus, P. xanthopus, P. personatus, P. protea, Trametes cingulata, T. badia, T. cubensis, T. Persoonii, T. floccosus, Fomes senex, F. pectinatus, Favolus scaber*) in addition to those mentioned above. In all these species I have found that, at the end of the rainy season and at intervals between two showers of rain, the basidia are themselves gradually transformed into hyphal elongations with terminal spores which are exactly like the basidiospores; in two instances (*viz. Trametes lactinea* and *Polystictus protea*) I noticed that there was copious spore-fall from such hyphal elongations on the surface of the agar in an agar plate. These elongations may be regarded as paraphysoid hyphae or cystidia with "pseudoconidia" or "basidioconidia" at the tips. Conversely, if showers of rain come in the middle of the dry season, the hymenial elongations soon become reconverted into basidia; a preliminary note has been published in *Nature* (Bose, **1940**).

Pieces of the sporophores of various Polyporaceae were attached to a lid of a petri dish containing 2.5 per cent agar, and the spore-deposit on the surface of the agar was examined daily. In many cases I have found that after the spore-fall has completely

FIG. 1.

ceased and the agar-plate has become comparatively dry, the pieces of sporophores when sectioned show pore-tubes in which basidia have been almost completely replaced by hyphal elongations, all these elongations having distinct clamp-connexions at their bases (FIG. 8: 1) and some bearing rudimentary spores at their apices (FIG. 8: 2). During the rainy season it is not uncommon to come

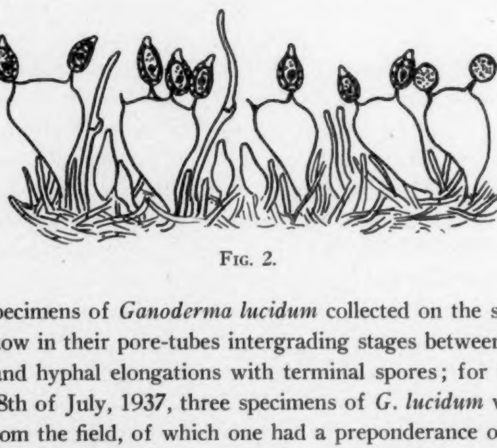

FIG. 2.

across specimens of *Ganoderma lucidum* collected on the same day which show in their pore-tubes intergrading stages between regular basidia and hyphal elongations with terminal spores; for instance, on the 28th of July, 1937, three specimens of *G. lucidum* were collected from the field, of which one had a preponderance of hyphal elongations with secondary spores (FIG. 1), while the other two had preponderance of basidia (FIG. 2, 3). This is probably be-

cause specimens growing in nature during the rainy season are not equally exposed to the rains. During the dry season (*e.g.* the months of May, September, October, November, December) normal basidia (10 to 11 μ broad) are often found in pore-tubes of specimens of *G. lucidum* and *G. applanatum* growing in nature, intergrading into beaked narrower basidia (4 to 9 μ broad) and finally into clamped and elongated hyphae with terminal mature brown spores which are indistinguishable from the normal basidiospores (FIG. 4). These represent gradual stages in the transformation of basidia into hyphal elongations with terminal spores, according to the season. On fixing a piece of fresh and moist sporophore of *Ganoderma* (*G. lucidum* and *G. applanatum*) from the field during the rainy season to the lid of an agar-plate placed

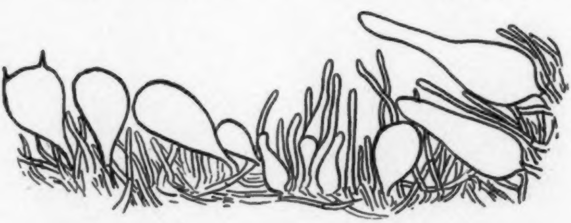

FIG. 3.

within a desiccator containing sulphuric acid at about 80 per cent relative humidity and slicing off a portion each day for examination, the gradual conversion of basidia into hyphal projections with terminal rudimentary spores could be distinctly followed in the course of the second or the third day (FIG. 5), but usually there was no spore-fall in such cases. Such conversion into hyphal elongations with clamp-connexions and terminal spores I could, by similar treatment, follow at about 80 to 85 per cent relative humidity in cases of fresh and moist specimens of *Polyporus ostreiformis, P. rubidus, P. luzonensis, Polystictus versicolor, Trametes cingulata, T. Persoonii, T. badia, Lenzites repanda* and *L. striata* collected from the field in July, August and September 1938 (*i.e.* the rainy season). The preliminary stages of gradual conversion of the normal basidium with four rudimentary sterigmata (FIG. 6 *a*) into narrow and short hyphal elongations are depicted in figure 6,

where it is shown how the basidial apices become narrower and the sterigmata become approximated as thinner and longer stalks bearing abortive spores (FIG. 6 *b–d*), ultimately only one of these stalks (FIG. 6 *e–h*) elongates, while others lag behind and are gradually shed.

On examination of a very large number of sections of fresh specimens of several Polyporaceae during the successive rains for a number of years since 1931, I find that it is mainly the water-relation which controls the transformation of basidia into hyphal elongations with clamp-connexions and terminal spores and its

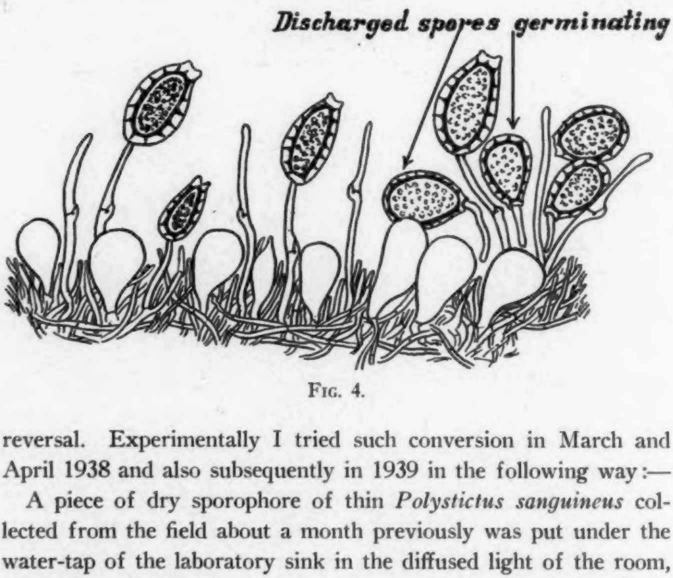

Fig. 4.

reversal. Experimentally I tried such conversion in March and April 1938 and also subsequently in 1939 in the following way:—

A piece of dry sporophore of thin *Polystictus sanguineus* collected from the field about a month previously was put under the water-tap of the laboratory sink in the diffused light of the room, the water dripping in a continuous shower for eighteen hours [1] through a perforated thimble attached to the water-tap. The sporophore originally had pore-tubes full of immature basidia (without sterigmata and spores), and in some pore-tubes there were a few hyphal elongations; but being kept under running water for eighteen hours the hyphal elongations entirely disappeared by way of gradual loss of protoplasm from the apex and of shedding of dead

[1] This period has been found to be different for different species.

parts (FIG. 7), and the majority of the pore-tubes developed mature basidia with sterigmata and spores (FIG. 8:3). Another piece of the same sporophore was attached to the lid of an agar-plate and was sectioned daily; for the first two or three days when the relative humidity [2] was about 97 per cent, there was copious spore-fall on the agar-surface and pore-tubes showed normal mature basidia ($10–12 \times 5–6\,\mu$) in a row; but as the water of condensation gradually disappeared from the plate and it became comparatively dry in the course of three or four days, the relative

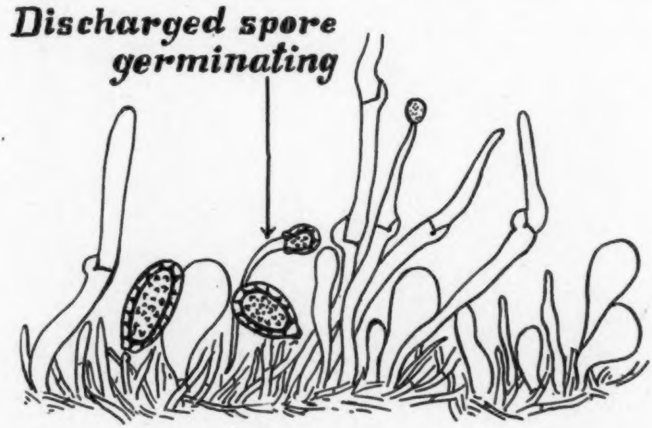

FIG. 5.

humidity being brought down to about 85 per cent, the spore-fall became scanty and pore-tubes displayed a development of a large number of clamped and elongated hyphae with terminal spores and of a few deformed basidia which were much longer and narrower than the normal ones (about 18 long and $2–3\,\mu$ broad) (FIG. 8:4, 5). Now the two pieces were reversed in position, *i.e.*, the one under running water was dried at room temperature for a day and

[2] The relative humidity of agar-plates and of the empty plate referred to in the subsequent part of this paper was determined by taking glass-jars into which the dew-point hygrometer could be introduced and by keeping the glass-jars in identical conditions as the plates; for this humidity-determination I am indebted to Dr. B. C. Basu of the Tropical School of Medicine of Calcutta.

was put inside an agar-plate, fixed to its lid for four days, and the other from the agar-plate was put under running water of the water-tap for a day. It was found that the piece under running water developed normal regular basidia (some with sterigmata and spores) in a row in the pore-tubes as in figure 8:3 without any hyphal elongations, while the other one inside the dry agar-plate

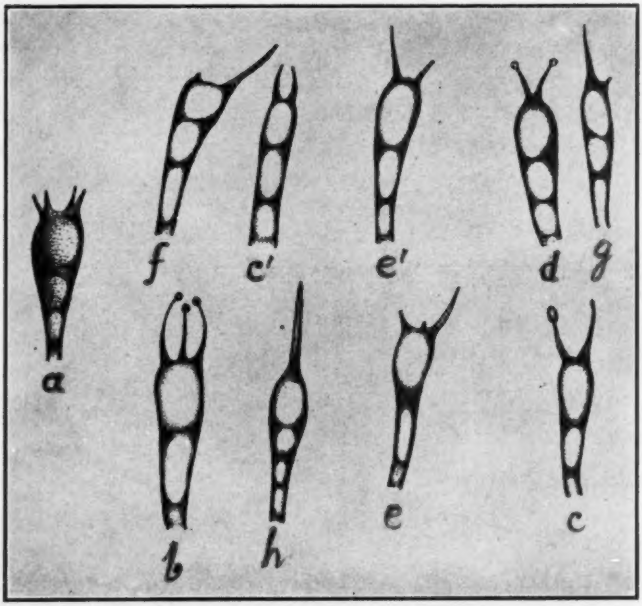

FIG. 6 *a–h*. Camera lucida sketch from two neighboring pore-tubes of the hymenial surface of *Polystictus versicolor* at about 80 per cent relative humidity under oil immersion lens with eye-piece no. 5, showing the preliminary stages of gradual conversion of the normal basidium with four sterigmata into narrow and short hyphal elongation.

showed in the pore-tubes hyphal elongations and elongated, narrower and abnormal basidia, some of the hyphal elongations having terminal spores. This experiment was repeated several times and sometimes in reverse order, and in each case the same result was uniformly obtained. The experimental conversion of basidia into hyphal elongations with clamp-connexions and with terminal spores

in some cases, and conversely, the conversion of hyphal elongations into basidia were quite successful with thin and dry specimens of *Polystictus sanguineus, P. hirsutus, P. versicolor, P. xanthopus, P. personatus, P. proteus, Polyporus rubidus, P. ostreiformis,* and *Trametes cingulata* collected from the field about a month previously. In each case the result was verified by several repetitions. The conversion did not succeed well with very thick or soft specimens of *Polyporus, Trametes, Lenzites, Daedalea, Fomes,* etc.;

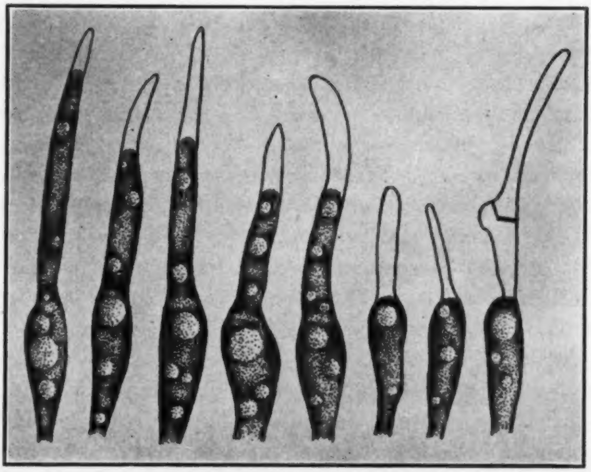

FIG. 7. Camera lucida sketch, under oil immersion lens and eye-piece no. 5, of hyphal elongations showing gradual loss of protoplasm from the apex.

and it was also noticed that if the spore-fall in moist condition within an agar-plate were too prolonged, the hyphal elongations could not be reconverted into basidia under the water-tap and the whole piece in such case decayed. In the case of *Ganoderma lucidum* and *G. applanatum,* however, success, *i.e.* the transformation of hyphal elongations into regular basidia in a row, was attained in October, 1935, by keeping the entire log with attached fruit-bodies under the water-tap for two days and in the case of *Polyporus grammocephalus* in September, 1939, for less than a day. If such sporophores are severed from the substratum and kept under running water, they undergo gradual decay. The degree of desic-

cation tolerated by various species, as is known, varies greatly; some like *Polystictus,* thin species of *Polyporus,* etc., appear capable of withstanding almost complete drying, while others, especially thick or soft specimens of *Fomes, Daedalea, Trametes, Lenzites,* etc., are not thus resistant. In this connection I have to record my personal experience of the revival of the fruit-body of *Polystictus sanguineus* after eight months' desiccation over pure sulphuric acid in a vacuum desiccator (*i.e.,* approximately at zero humidity). A piece of this desiccated sporophore, when put inside a moist agar-plate, soon began throwing out spores and continued to do so for seven days; the spores germinated easily and the germinating hyphae formed clamp-connexions in the course of two days and advanced to full development in tubes of malt-extract agar. It was further noted that a piece of the sporophore of *Polystictus sanguineus* that was kept inside a sterilized plate (without agar and at a relative humidity of about 53 per cent) even after seven days did not show any change. Similarly, specimens kept at 72 per cent and 62 per cent relative humidity over suitable concentrations of sulphuric acid for about a month, and also those kept at zero humidity as described above for *P. sanguineus,* showed no transformation and did not deposit any spores. Evidently, the humidity had been too low to permit of the conversion being effected.

The experiments just described undoubtedly give a clue to what happens under natural conditions; it indicates that, in many Polyporaceae, the amount of rainfall governs the transformation of basidia.

Regarding polymorphism of basidia, Patouillard (**1880**, **1881**) recorded that in *Pleurotus ostreatus,* besides normal basidia with four sterigmata and spores, the hymenium shows cystidia with one terminal oval or globular spore or conidium. In a short paper (**1889**) he gave it as his opinion that every cell of a fruit-body is a potential basidium and, by comparison of the upper surface of the pileus with the hymenium, found complete homology between the basidia and spore-bearing hairs. Matruchot (**1897**) observed in the same species the intergrading series, numerically balancing one another, between the two preceding categories of structures observed by Patouillard, and thought that the normal basidia, hy-

menial cystidia with 1, 2 and 3 sterigmata, and extra-hymenial cystidia with pseudoconidia are but forms of one and the same type. Richard Falck (**1909**) held that in *Lenzites* the fertile hyphae, if they do not attain to basidia-formation, become transformed into vegetative hyphal threads or hairy hyphae. In the hymenium of *Spongipellis Litschaueri* Lohwag (**1931**) observed between the normal basidia (4–5 μ broad) bearing spores thin hyphal threads comparable to "paraphysoid hyphae," which are 2 μ broad. He (**1937**, p. 165) cited a number of instances, observed by other workers besides himself, where basidia are intermixed with such hyphal hairs in the hymenial layer. He regards them as examples of reversion of basidia to vegetative paraphyses. But these hyphal threads or hairy hyphae were apparently without any terminal spore. Corner (**1934**) held that in the fruitbody of *Collybia apalosarca* "almost every apical cell on cessation of growth enlarges into a basidium or a cystidium." Heim (**1933**) found in the pore-tubes of *Xanthochrous Patouillardi* Rick. var. *congoensis,* besides normal basidia with four sterigmata, elongated hyaline tramal hyphae bearing at their apices spores indistinguishable from the normal basidiospores. Heim and Malençon (**1933**) held that in *Lycoperdellon Torrendii* Bres., where basidia and basidiospores are not formed under favorable circumstances, "basidioconidia" which are different from the real conidia, usually appear; but they did not investigate further the nature of the unfavorable circumstances. At one place (at p. 14) they surmise that such secondary basidiospores ("basidioconidia") probably arise as a result of the functional derangement of the basidium, especially its nuclear phenomena. In the case of *Ganoderma,* I (**1935**) have found that the nucleus of a secondary basidiospore arises by amitosis from the fusion-nucleus of the apical cell of the hyphal elongation, and I have illustrated the successive stages as seen in fixed and stained preparations. By growing *Polyporus squamosus* in complete darkness Oehm (**1937**) obtained an irregular chlamydospore-fruitbody in which basidia failed to develop but hyphae bore chlamydospores, sometimes in chains, with clamy-connexions; these spores were brown, bigger than the normal basidiospores, and did not germinate in the various media tried. Such sporophores were regarded as sterile, resembling outwardly the dark form of *Lentinus squamo-*

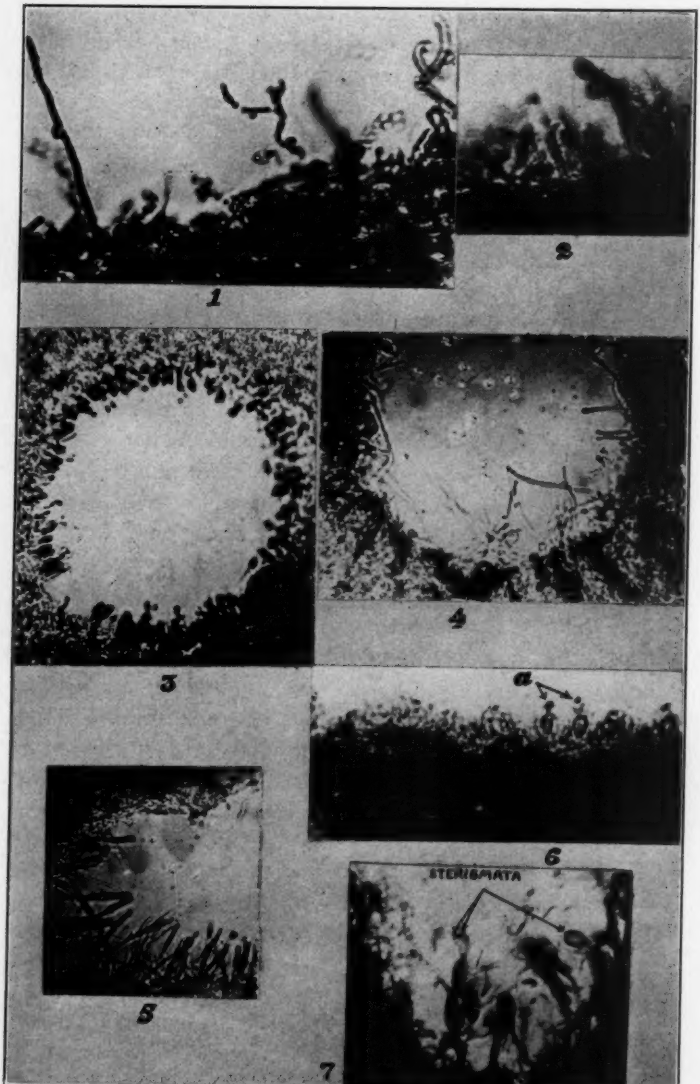

FIG. 8.

sus. In some members of the Agaricaceae French mycologists, such as Josserand (**1937**) and others, have from time to time in the *Bull. Soc. Myc. France,* described and illustrated probasidia, various forms of cystidia (cheilocystidia, pleurocystidia, etc.) and basidial proliferations mixed with the normal basidia as hyphal elongations; but these structures are never terminated by secondary spores.

The workers cited above have in no instance correlated this change of form of basidia with varying external conditions, that is, they have not noted the external conditions under which such change takes place. In India where, unlike Europe, there is usually a well-marked rainy season, I have readily followed these changes during the successive rains since 1931, and the water-tap experiment and the "agar-plate-technique" have fully corroborated my field observations. I have always found that in the pore-tubes where there is a preponderance of basidia the hyphal elongations are few, and that when the hyphal elongations preponderate basidia are very scarce.

Montgomery (**1936**) obtained in sections of the pore-surface of *Fomes fraxineus* in culture typical basidia bearing four basidiospores. "This," he states, "is contrary to the observations made in the same species by Baxter (**1925**) who reports that only secondary spores were produced in the pores. None of these secondary spores were seen by the author." This apparent contradiction between the observations of Baxter and of Montgomery on *Fomes*

FIG. 8. All the microphotographs were taken under Zeiss apochromat 2 mm. oil-immersion lens N.A.1.4 and eye-piece no. 5. 1, pore-tube of *Polystictus sanguineus* showing a large number of hyphal elongations with clamps; 2, hyphal elongations with terminal rudimentary spores in the pore-tube of *Polystictus hirsutus;* 3, pore-tube of *Polystictus sanguineus* kept under running water for 18 hours showing development of mature basidia with sterigmata and spores and almost total disappearance of hyphal elongations; 4, transverse section of a pore-tube of *Polystictus sanguineus* from dry agar-plate showing development of a large number of clamped and elongated hyphae and of some deformed (narrower and longer) basidia; 5, longitudinal section of a pore-tube of *Polystictus sanguineus* from the dry plate as in figure 4; 6, hymenial layer of an abnormal *Ganoderma lucidum* showing brown thick-walled basidia some of which have rudimentary spores (a) emerging out of a germ-pore-like area; 7, portion of a pore-tube of an abnormal *Ganoderma lucidum* showing elongated, thick-walled and brown basidia with vacant sterigmata.

fraxineus (not available in India) admits, in view of my experiences with more than a dozen Polyporaceae, an easy explanation. Neither Montgomery nor Baxter recorded the condition in their respective culture-tubes. It is, therefore, very likely that Montgomery's culture-tubes were in a moist condition when the pore-tubes would be full of basidia, while Baxter's had been kept comparatively dry when basidia would be replaced by hyphal elongations.

Incidentally, in some specimens of *Ganoderma lucidum* growing on roots of the stump of a dead coconut palm in August 1937 and July to October 1938 at Dum Dum (Calcutta), and on the trunk of a living *Diospyros embryopteris* tree at Belgachia (Calcutta) in August 1938, I observed some *pseudobasidia* in the hymenial layer similar to those figured and described by Heim (**1932**, **1932**(a)) in *Podaxis indicus* and *Podaxis aegyptiacus* and cited by Lohwag (**1936**). They were thick-walled and brown in colour like the spores situated among the normal basidia and had at the apex, in some cases, a thin area like a germ-pore through which a rudimentary spore emerged (FIG. 8:6), while others had vacant sterigmata (FIG. 8:7). Heim regards such pseudobasidia or hypertropied basidia as equivalent to macrospores or giant spores. According to him their formation is due to acceleration of sporulation under the influence of unfavorable climatic or nutritive conditions, resulting in the formation of spores before the maturation of basidia. Unfortunately, the conditions to which he refers are vague and indefinite.

SUMMARY

Basidia of many Polyporaceae at the end of the rainy season and during intervals between two showers of rain have been observed to be gradually transformed into hyphal elongations with terminal spores that are exactly like basidiospores. Conversely, on the advent of rain in the middle of a dry season, the hyphal elongations soon become reconverted into regular basidia.

Such conversion has been reproduced under experimental conditions. When a piece of the fruitbody of a thin and desiccated specimen was placed under the running water of a laboratory-tap overnight, the hyphal elongations entirely disappeared from the

pore-tubes and the majority of the pore-tubes developed mature basidia with sterigmata and spores. When a piece of the same fruitbody was stuck to the lid of a moist agar-plate and examined daily under the microscope, it was found that, as the water of condensation gradually disappeared from the plate in the course of three or four days and the plate became comparatively dry (the relative humidity becoming reduced to about 85 per cent), the pore-tubes displayed a development of a large number of clamped and elongated hyphae with terminal spores and a few abnormal elongated narrower basidia. The experiment was repeated several times and sometimes in reverse order, and in each case the same result was obtained. Such experimental conversion was quite successful with thin and easily desiccated specimens of *Polyporus, Polystictus,* and *Trametes.* It did not succeed well with very thick or soft specimens of *Polyporus, Trametes, Lenzites, Daedalea, Fomes,* etc., as it is known that all specimens cannot withstand desiccation to the same degree. This experiment confirms my observation that it is mainly the water-relation which controls the transformation of basidia into hyphal elongations with clamp-connexions and terminal spores and *vice versa.* None of the previous workers (Patouillard, Matruchot, and Heim) have correlated this change of form of basidia with the varying external conditions.

In some specimens of *Ganoderma lucidum* collected in 1937 and 1938, brown thick-walled basidia resembling those described by Heim in *Podaxis indicus* and *Podaxis aegyptiacus* under the name of *pseudobasidia,* were found in the hymenial layer.

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DISTRIBUTION OF ANTAGONISTIC FUNGI IN NATURE AND THEIR ANTIBIOTIC ACTION [1]

Selman A. Waksman and Elizabeth S. Horning

(with 7 figures)

The ability of many fungi to antagonize various microörganisms, notably bacteria and other fungi, has been well established, as brought out in several recent reviews (**6**, **11**). In most cases, attention has been centered upon the antagonistic action of various fungi against organisms capable of causing plant diseases (**4**, **6**, **15**, **8**). Fungi capable of antagonizing pathogenic organisms belonging to the groups *Ophiobolus, Rhizoctonia* and *Fusarium* have been studied most extensively.

It has been demonstrated in recent studies that fungi capable of inhibiting the growth of bacteria are distributed among various taxonomic groups. The following organisms have so far received the greatest attention as agents possessing bacteriostatic and bactericidal properties: 1. The *Penicillium notatum* group, from which Fleming (**3**) and, later, other British investigators (**9**, **1**) isolated an active substance, designated as penicillin; 2. other species of *Penicillium,* including *P. citrinum* and *P. cyclopium,* from which Raistrick and associates (**10**) isolated respectively *citrinin* and *penicillic acid,* substances possessing some antibiotic properties; 3. the *Aspergillus flavus* group, from which White (**16**) and Glister (**5**) isolated active substances; 4. the *Trichoderma* and *Gliocladium* groups, from which Weindling (**15**) isolated an antibiotic substance designated as *gliotoxin.*

In spite of the fact, however, that many fungi were thus found to be capable of depressing and even gradually destroying the growth of many bacteria, no systematic study has so far been made of the occurrence and distribution of such antagonistic or-

[1] Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Soil Microbiology.

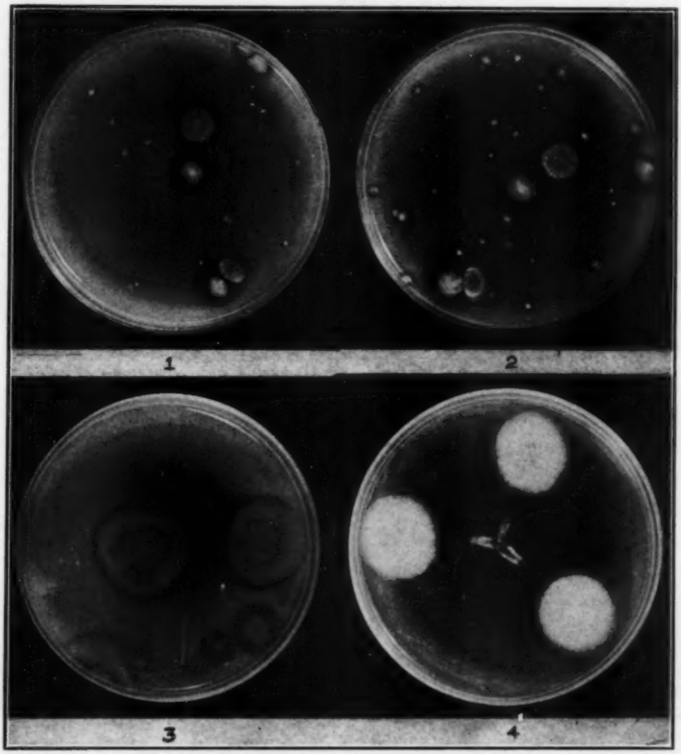
ganisms in nature. Virtually all previous investigations were based either upon chance contaminants developing on bacterial plates exposed in the laboratory or upon results obtained by testing cultures taken from collections. The wide distribution of antagonistic bacteria and actinomycetes in natural substrates, such as soils and composts, suggested the possibility that antagonistic fungi also occur in these substrates. This and the fact that their development might be stimulated by various treatments led to a systematic survey of the occurrence and activities of these organisms.

SUBSTRATES FOR THE ISOLATION OF ANTAGONISTIC FUNGI

When bacteria are added to soil, to sewage or to composts of plant residues, they are rapidly destroyed by antagonistic organisms, which are able to develop at their expense (**2**, **12**, **13**). Under natural conditions, these substrates continuously receive large numbers of bacteria, either by the introduction of foreign materials rich in bacteria (animal droppings, stable manure, garbage), or as a result of the stimulated multiplication of bacteria caused by various treatments. One would expect, therefore, that stable manures and soil should be the logical sources for the isolation of antagonistic organisms, including fungi, especially since these organisms grow much better in soils and in composts than, for example, in sewage. These substrates were largely used, therefore, in this investigation. A series of soils, differently treated with manures and fertilizers, were selected from the experimental fields of the New Jersey Agricultural Experiment Station; stable manures and composts prepared from various plant materials were also employed.

METHOD OF ISOLATION OF ANTAGONISTIC FUNGI

The selection of suitable methods for the isolation of antagonistic organisms is very essential. If a material, such as soil, sewage or manure, is plated out on ordinary organic or synthetic media, in the dilutions commonly employed for the enumeration or isolation of bacteria, the chances of obtaining fungi, especially antagonistic forms, are rather remote. When these media are acidified to a pH of 4.0 or 4.5, the development of the majority of bacteria is



FIGS. 1–4. Isolation of antagonistic fungi and testing their anti-bacterial properties.

inhibited, but most of the fungi are still able to grow. By using these acid media, it is possible to plate out lower dilutions, enabling fungi to develop without the numerous bacterial colonies obtained on the ordinary plates. After 2 days incubation at 28° C., the fungi can readily be isolated from the colonies formed on these plates. Since there is no evidence that these fungi possess properties antagonistic to bacteria, all the colonies must first be isolated and tested. This tends to make the procedure both tedious and difficult, without the required assurance that many antagonistic types will be obtained. Fortunately, other and more suitable methods have been developed and can now be employed.

The following general procedures for the isolation of antagonistic fungi from soils and other materials have been utilized in this investigation: 1. Suitable agar plates are seeded with various bacteria; these are allowed to develop for 12–24 hours, and the plates inoculated with small particles of soil or manure. 2. Washed suspensions of different living bacteria are added to washed agar containing a carbohydrate and some phosphate; this bacterial agar is used for plating out the soil or the manure. The following modification of the second procedure was used in most of this work. Two to three day old cultures of *Bacillus subtilis, Sarcina lutea, Staphylococcus aureus* and *Escherichia coli* grown on agar or in liquid media are suspended in sterile water, centrifuged, washed, again centrifuged and fairly heavy suspensions of the bacteria in sterile water added to washed agar, enriched by 1 to 3 per cent glucose and 0.05 to 0.1 per cent KH_2PO_4. The reaction of the medium is thus slightly acid, making it highly favorable to the development of antagonistic fungi. The soil or manure is plated out on the above agar in dilutions of 1 : 500, 1 : 2,000 and 1 : 10,000, and the plates are incubated for 2–4 days at 25–28° C. The antagonistic fungi produce colonies surrounded by clear zones, as shown in figures 1–2, as a result of the dissolution of the bacteria on the plate. These colonies are picked, by cutting out bits of mycelium, and transferred to plates of sterile glucose-peptone agar.

Before a specific fungus culture is designated as an antagonistic organism, it is further tested by two specific methods: (*a*) The pure culture is inoculated upon the washed agar medium enriched with living *S. lutea* or *B. subtilis* cells; cultures possessing antagonistic properties are surrounded by clear zones, free from bacterial cells (FIG. 3). (*b*) Two to four fungi are "spot" inoculated upon solidified glucose-peptone agar plates and incubated at 28° C., for 20 to 24 hours, to enable the spores of the fungi to germinate or the mycelium to develop and form small colonies; an aqueous suspension of *B. subtilis* or *Staphylococcus aureus* is streaked from the center of the plate to the edge of the growing colony and the plates are incubated for 24 hours at 28° C. if *B. subtilis* is used or 37° C. if *S. aureus* is used; the formation of antibiotic substances by the fungus is detected by the presence of a clear zone between

the fungus colony and the growth of the test organisms (FIG. 4). The clear zones vary in width from 1 mm. to 3 cm. Those fungi which produce the widest zones of bacterial inhibition or of bacteriolysis and thus appear to be decidedly antagonistic are selected for further studies.

These procedures for the isolation and testing of antagonistic fungi proved to be very convenient, especially when it was necessary to isolate and test a large number of fungi within a short time. It is, of course, possible that the fungi which showed only limited antagonism to the particular test bacterium may prove to be much more active against other organisms, because of the known selective action of microbial antagonists. The use of other media might have modified the nature of the fungi thus obtained. In spite of these limitations, however, it was possible to isolate a large number of fungi which possess anti-bacterial properties in varying degrees.

The fungus colonies that were thus selected for antagonistic properties by one of the above methods, were now isolated from the test plates and transferred to sterile agar media. In many cases, the cultures thus obtained were not pure, but represented mixtures of two or more different organisms. These were now separated by replating and isolating individual colonies. The fresh cultures were often retested for their antagonistic action.

THE PRODUCTION OF ANTIBIOTIC SUBSTANCES BY ANTAGONISTIC FUNGI

After the fungi have been isolated from the plate and grown in pure culture, they were studied further for the production of antibiotic substances in liquid media. Various organic and synthetic media were used for this purpose. The one most commonly employed is the nitrate-glucose solution, or the so-called Czapek-Dox medium. Although the addition of yeast extract, corn steep liquor, or brown sugar was found to be highly favorable to the production of penicillin and some other antibiotic substances, in most cases they were quite superfluous or even injurious to the production of various other antibiotic bodies.

A shallow layer of medium (60–100 ml. per 250 cc. Erlenmeyer flask or 100–250 ml. per 1 liter flask) was found to be most satis-

factory. In some cases, however, deeper layers resulted in the production of some rather interesting antibiotic substances. It was found, for example, that certain strains of *P. notatum* produce a factor active against *E. coli* when grown in much deeper layers of medium (150–175 ml. per 250 cc. flask or 600–700 ml. per 1 liter flask). The cultures are usually incubated, for 7–10 days, at 25–28° C. Because of differences in the rates of growth of various antagonistic organisms upon different media, the optimum period of incubation may vary from 4 to 15 days. This is particularly important if the fact is recognized that different fungi may produce several active substances, selective in their action against various bacteria. These substances need not appear simultaneously in the medium, but may appear and disappear at different periods of incubation.

The culture filtrates of the antagonistic fungi are now tested for antibiotic activity, by one of several procedures: 1. different volumes (1.0–0.001 ml.) of the filtrates are added to 5 or 10 ml. portions of sterile liquid broth; this is inoculated with the test organism and incubated. Absence of growth of the test organism, as determined by streaking, or by plating on sterile agar media, or by a change in turbidity, is taken as the end point; 2. varying amounts of the filtrate of the antagonist are incorporated into nutrient agar and the plates are streaked with 2 to 4 test organisms. Positive or negative growth of these give the limit of activity or the concentration of the active substance in the filtrate; 3. solid media are inoculated with one test organism and treated with varying amounts of the active filtrate; this is either incorporated directly into the medium or allowed to diffuse through it from a central point. The reduction in the number of bacterial colonies or the width of the zone of inhibition of bacterial growth is taken as a measure of the activity of the culture filtrate.

Each of the above methods has its advantages and disadvantages. For the study of substances which have bacteriolytic properties, the first is most convenient. Sterility of the materials used in making this test is essential. The second method is most convenient for a rapid survey of a number of organisms. It has the added advantage that several test organisms can be streaked on the same plate; however, it does not yield accurate quantitative results. The third

method, of which several modifications exist, such as the Oxford method (1), readily lends itself to a quantitative expression of the concentration of the active substance and does not require sterile material. Only one test organism can be used, however, and the method itself is rather cumbersome.

Since it was essential, in carrying out this survey of the occurrence of antagonistic fungi, to determine the specific effects of a large number of fungi against a variety of bacteria, the second method was found to be most suitable. Since most of the antibiotic substances produced by microörganisms are selective in their action, not only against gram-positive vs. gram-negative bacteria, but also against specific organisms within each group, this method proved to be particularly useful. In most instances, four test organisms were used in determining the antibiotic activity of the unknown active substance in each culture filtrate. The units of activity can best be expressed by the ratio of the dilution of the culture filtrate added to the agar to the volume of agar used in the plate. The highest dilution which gives complete inhibition of growth of the test organism was taken as the end point. If growth was not fully but only partly inhibited, the inhibiting dilution was interpolated between that giving reduced growth and that giving no growth at all. The period of incubation of the plates was found to be significant. Ordinarily, 24 hours incubation at 28° C. was used for *B. subtilis, B. mycoides, E. coli,* 24 hours at 37° C., for *S. aureus,* and 48 hours at 28° C., for *S. lutea.* In most cases, the tests did not change on further incubation of the plates, though in some cases the activity tended to be reduced or even completely disappeared when the plates were incubated for a day or two longer. This points either to the destruction of the active substance on continued incubation, or to the gradual adaptation of the test organism to the substance. Certain fungi tend to produce substances that are active for only a short time.

ISOLATION OF SPECIFIC ANTAGONISTIC FUNGI

By the use of the above methods, 160 cultures of fungi were isolated in our laboratory from a number of soils and composts. Many of these fungi were found, by the streak method, to be active but did not produce any soluble active substances when grown in

liquid culture media. This may be due to the fact that either these organisms produce antagonistic effects only in the presence of the living antagonists or that favorable conditions for the production and accumulation of the antibiotic substance by these organisms have not been attained. Cultures 94, 96 and 118 are specific illustrations of that effect. It was later found that, in some cases at least, the modification of the liquid medium or of the condition of growth resulted in the production of an active substance.

The various antagonistic fungi thus far isolated in this survey can be divided into 9 distinct groups, on the basis of the taxonomic position of the organisms. They also appeared to vary greatly in the nature of their antibiotic activity. These groups can be briefly listed as follows:

Group 1. *Chaetomium* group. Only one organism, an unidentified species of the genus *Chaetomium,* was found to belong to this group. It is listed as No. 1. The reason for creating a separate group for this one culture was governed by its systematic position.

Group 2. *Aspergillus fumigatus* group. This group was found to comprise a large number of strains, isolated from different substrates. These strains were found to possess antagonistic properties varying considerably in degree. Morphologically, the various strains did not appear to be very different. Some were antagonistic not only to bacteria but also to certain fungi. Fifteen strains belonging to this group have thus far been isolated, namely, Nos. 14, 20, 26, 35, 84, 85, 87, 88, 92, 93, 97, 101, 102, 106, 107.

Group 3. *Aspergillus clavatus-A. glaucus* group.[2] Two strains of *A. clavatus* were isolated from manure composts, namely, 129 and 130, differing somewhat in activity. One strain of *A. glaucus* was isolated from the soil.

Group 4. *Aspergillus flavus* group. Among the many strains of fungi thus far isolated, only one appeared to fall into this group (No. 136). The organisms of White (**16**) and of Glister (**5**) also belong to this group.

Group 5. *Penicillium luteum-purpurogenum* group. A number of species of *Penicillium* were isolated and found to possess an-

[2] Dr. E. L. Spencer assisted in the isolation of this group, as will be reported elsewhere.

tagonistic properties. The various cultures could be divided, for convenience, into two groups; namely, 5 and 6. Group 5 comprises the following strains: 12, 108a, 108c, 109, 110, 111, 113, 114, 115, 116, 118, 119, 125, 140, 142, 146, 126, 132, 134, 138.

Group 6. Green-*Penicillium* group. This is a large heterogeneous group of organisms, including a number of forms varying greatly in activity, namely, Nos. 94, 96, 99, 108b, 117, 120, 121, 123, 124, 126, 127, 128, 132, 134, 135, 138, 139, 141, 161, 162, 163. The highly active *P. notatum* also belongs to this group. Three strains (W, F, O) of this organism were obtained from various laboratories and may also be listed here.

Group 7. *Trichoderma* group. Although members of this group were found by Weindling (**15**) to comprise organisms with pronounced antagonistic properties, especially active against fungi, only two strains were isolated in this study (Nos. 86, 160). These strains were not found to be very active against the test bacteria, at least under the conditions of study. No attempt was made to create special conditions for the production and isolation of the active substance.

Group 8. *Fusarium-Cephalosporium* group. The organisms belonging to this group did not readily produce any antagonistic substances when grown upon the liquid medium. Some of the cultures isolated, however, appeared to be decidedly antagonistic. This antagonistic effect often disappeared after prolonged incubation of the test organisms. The following isolated strains were found to belong to this group: Nos. 82, 83, 90, 91, 95, 98, 100, 103, 104, 105, 131, 133, 137, 143, 144.

Group 9. Miscellaneous group. A number of other organisms with various antagonistic properties were isolated from soils and composts. They have either been only incompletely identified or were insufficiently studied.

It is of interest to note that none of the Phycomycetes is included among the above groups. No species of *Mucor* or *Rhizopus* were so far isolated as antagonists by the use of the above methods. Six cultures of known species of *Rhizopus* were tested for antagonistic properties by the plate method, using *B. subtilis* as the test organism. They all gave negative results. No species belonging to the Basidiomycetes were isolated on the plate, possibly

for the simple reason that the above method did not readily lend itself to the development of these organisms.

A number of the fungi thus isolated and representing the various groups of antagonists were now tested for the production of antibiotic substances in liquid media. The results, reported in Table 1, show that these antagonistic fungi vary considerably in activity, at

TABLE 1

ANTIBIOTIC ACTIVITY OF DIFFERENT FUNGI FRESHLY ISOLATED FROM SOILS AND OTHER NATURAL SUBSTRATES

Group No.	Organism	Age of culture, days	pH	Activity[2]		
				B. mycoides	*B. subtilis*	*S. lutea*
1	*Chaetomium* sp.	6	6.0	6	100	250
1	*Chaetomium* sp.	9	6.3	3	20	>300
2	*A. fumigatus* 20	9	7.8	>30	10	>300
2	*A. fumigatus* 35	9	7.6	>30	6	>300
2	*A. fumigatus* 84	9	7.8	100	30	>300
2	*A. fumigatus* 93	9	7.9	60	30	>300
2	*A. fumigatus* 107	9	7.5	>100	30	>300
3	*Fusarium* sp. 95	9	4.5	0	0	Tr.
3	*Fusarium* sp. 103	7	7.1	0	Tr.	Tr.
4	*P. luteum-purpurogenum* 108	6	3.8	10	20	50
4	*P. luteum-purpurogenum* 12	8	—	3	10	10
4	*P. luteum-purpurogenum* 113	8	—	3	10	15
4	*P. luteum-purpurogenum* 116	8	4.8	6	6	10
4	*P. luteum-purpurogenum* 119	8	3.5	0	3	10
5	Green *Penicillium* group 99[1]	8	—	10	200	60
5	Green *Penicillium* group 115	8	—	0	20	20
5	Green *Penicillium* group 117	8	—	6	10	20
5	Green *Penicillium* group 121	8	—	13	30	—

[1] After 48 hours incubation of test organisms little inhibiting effect was produced.

[2] Unit of activity = $\frac{10}{\text{Amount of culture necessary to inhibit growth of test organism in 10 ml. nutrient agar}}$.

least in their ability to produce active substances in a given medium. Very few of the culture filtrates of these fungi were active against *E. coli.* However, when the active substances produced by some of these organisms were concentrated, more were found to be active against *E. coli* and other gram-negative bacteria than was indicated by the original culture filtrate.

The various antagonistic fungi thus isolated from soils and composts were found to differ markedly in activity, qualitatively as well as quantitatively. Some of the strains, such as Nos. 20 and 84,

were much more active against *B. mycoides* than against *B. subtilis;* others, such as 108a and 132, were inactive against *B. mycoides,* or were less active against this spore-former than against *B. subtilis.* Some were found to be only weakly antagonistic, whereas others were very strong antagonists. Some produced antibiotic substances which could readily be isolated from the medium by the use of various solvents; with others, ordinary extraction or adsorption procedures have given rather unsatisfactory results.

STRAIN SPECIFICITY

Many of the antagonistic fungi freshly isolated or obtained from other laboratories (such as the *P. notatum* strains) were found to comprise strains or varieties which varied considerably in their antibiotic activity. This was also true of strains isolated from the same mother culture. Some of the strains proved to be completely inactive; other strains, though possessing the same general characteristic antagonistic properties, varied greatly in certain qualitative and quantitative respects. The organism studied most extensively from this point of view was *P. notatum.* The tendency of this organism to produce variants that differ in activity has been the cause of considerable trouble in the practical production of penicillin.

Eight different strains of *P. notatum,* available in England and in this country, were collected and tested by the various methods described above. These strains differed greatly in capacity to produce the active antibiotic substance, which may or may not have been true penicillin. In order to illustrate the variation in the results obtained, only three strains need be discussed here:

1. A pre-Fleming strain of *P. notatum,* probably the original isolation made by Westling. It was received from the latter by Dr. Thom and sent by him to the Centralbureau in Holland. From there, it came, in 1925, to Dr. Raistrick in England, was deposited by him in the Lister collection in London, and was received from Dr. St. John Brooks in 1940. This strain is designated as W.

2. The original Fleming strain of *P. notatum* received from Dr. St. John Brooks in 1940, and designated as F.

3. A post-Fleming strain of *P. notatum,* presumably isolated from Fleming's original culture, brought to this country by Dr. Florey and Dr. Heatley, and obtained from the latter. This strain is designated as O (Oxford strain).

The results of a few typical experiments are reported here, in order to illustrate the differences in behavior of the different strains

TABLE 2

INFLUENCE OF COMPOSITION OF MEDIUM UPON THE ACTIVITY OF FILTRATES OF *P. notatum*

Treatment of medium	Culture of *P. notatum*	Tested by plate method[1]							
		E. coli	*B. mycoides*	*B. subtilis*	*S. lutea*	*S. aureus*[2]			
						W	H	Dp	Df
$CaCO_3$	W	20	30	100	30	30	30	30	70
CSL[3]	W	0	25	30	30	30	70	30	70
$CaCO_3$	0	0	15	30	10	10	10	10	30
CSL	0	0	30	100	100	100	200	100	100
CSL + $CaCO_3$	0	0	100	100	100	80	200	80	150

[1] See table 1, footnote 2.
[2] Four different strains used as test organisms.
[3] CSL = Corn steep liquor, 6 cc. per liter.

TABLE 3

INFLUENCE OF AMENDMENTS UPON THE ANTIBIOTIC ACTIVITY OF 2 TYPES OF *P. notatum*

Supplement	Age of culture, days	Strain W				Strain 0			
		Test organism							
		E. coli	*B. mycoides*	*B. subtilis*	*S. lutea*	*E. coli*	*B. mycoides*	*B. subtilis*	*S. lutea*
No Ca	4	10	30	100	—	—	—	—	—
No Ca	9	10	10	30	30	0	10	10	10
$CaCl_2$[1]	4	25	30	80	—	—	—	—	—
$CaCl_2$	9	0	10	80	30	0	10	10	10
$CaSO_4 \cdot 2H_2O$	4	25	25	80	—	—	—	—	—
$CaSO_4 \cdot 2H_2O$	9	0	15	100	10	0	10	10	10
$CaCO_3$	4	25	30	100	—	—	—	—	—
$CaCO_3$	9	20	30	100	30	0	15	30	10
CSL[2]	4	0	8	10	—	—	—	—	—
CSL	9	0	25	30	30	0	30	100	100
CSL + $CaCO_3$	4	0	3	30	—	—	—	—	—
CSL + $CaCO_3$	9	0	15	30	30	0	100	100	100

[1] 0.5 gm. of salt per flask, for all salts.
[2] CSL = Corn steep liquor, 6 cc. per flask.

of *P. notatum* (tables 2, 3). The effect of supplementary treatments upon the production of the active substance was found to be dependent upon the strain of the organism employed. The two strains of *P. notatum* varied not only in the amount of active substance produced but also in the nature of its activity. This is shown by the fact that, whereas the W strain produced activity against *E. coli* under certain conditions, the O strain had no activity at all against this organism. Corn steep liquor greatly improved the activity of strain O but had little effect on the general activity of strain W; however, it depressed the production of the substance against *E. coli* by this strain. The nature of the calcium salt greatly modified the nature and yield of the active substance.

TABLE 4
EFFECT OF VOLUME OF MEDIUM ON ACTIVITY OF SEVERAL REPRESENTATIVE FUNGI

Culture No.	Volume of medium per 1 liter flask	Activity[3]			
		E. coli	*B. mycoides*	*B. subtilis*	*S. lutea*
1[1]	100	0	20	20	600
20[1]	100	0	300	150	800
20	300	0	300	60	800
84[1]	100	0	600	300	>1,000
84	300	0	300	100	>1,000
108*a*[1]	100	0	0	0	0
108*a*	300	0	0	20	10
P. notatum F[2]	100	0	3	15	—
P. notatum F	700	10	10	>100	—
P. notatum W[2]	100	0	45	70	70
P. notatum W	700	100	80	450	150

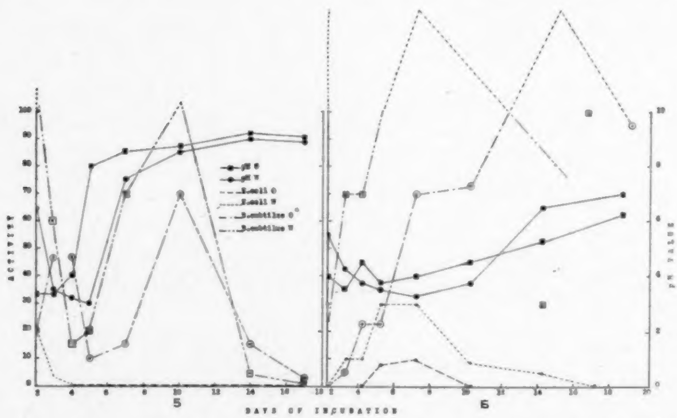
[1] Six days incubation at 28° C.
[2] Five days incubation at 28° C.
[3] See footnote 2, table 1.

INFLUENCE OF CONDITIONS OF NUTRITION UPON THE PRODUCTION OF ANTIBIOTIC SUBSTANCES

Among the various factors influencing the production of antibiotic substances by fungi, none are more important, next to the nature of the organism and the specificity of the strain, than the conditions of nutrition. A detailed study was made of the effect

of various mineral salts upon the production of antibiotic substances by *P. notatum* which may only be summarized here. The presence of iron was found to be favorable to both the *E. coli* factor and the general antibiotic activity of the culture. Zinc, however, even in concentration of 10 mg. $ZnSO_4.7H_2O$ per liter, greatly reduced the antibiotic activity of the culture as a whole and completely repressed the activity against *E. coli*. Manganese had little effect.

The effect of volume of medium in which the organism was grown is illustrated in table 4. Strains F and W of *P. notatum*



FIGS. 5–6. Course of formation of antibiotic substances by two strains of *P. notatum* (W and O). 5, in shallow, 0.6 cm., layers; 6, in deep, 4.5 cm., layers.

were the only ones that produced an active substance against *E. coli* in the culture filtrate, and they produced it only when grown in deep layers of medium. The activity of two of the strains of *P. notatum* and of culture 108a against all organisms was greater in the deeper layers. Cultures 20 and 84 (*A. fumigatus*), on the other hand, showed greater activity when grown in shallow layers.

Other factors were found to have an important influence upon the production of antibiotic substances by various fungi. It is sufficient to mention the effects of temperature and length of incubation period. Whereas many grew well at 37° C. and even at

50° C., the optimum temperature for production of the antibiotic substances appeared to be 28° C. or even less. Many produced the antibiotic substance at an early stage of growth, namely in 2–5 days, others required a longer incubation period for the production

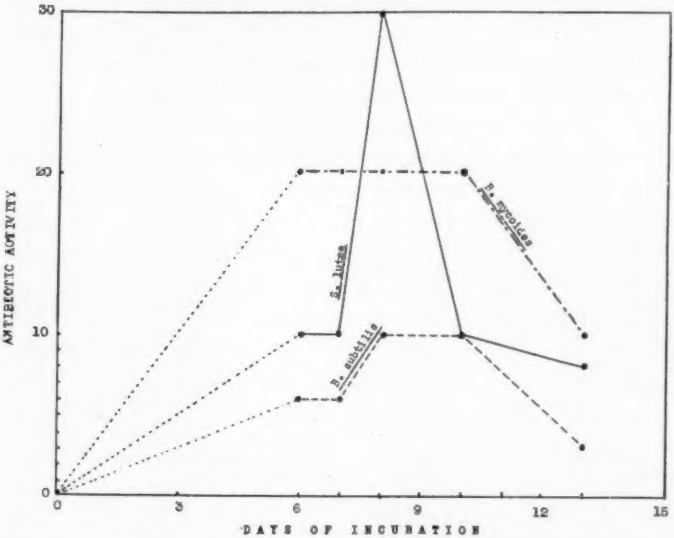


FIG. 7. Course of formation of the antibiotic substances by *Aspergillus fumigatus*. Each division represents 10 *B. subtilis* or *B. mycoides* units and 100 *S. lutea* units.

of the substance. In the case of *P. notatum* W, for example, the *E. coli* factor appeared in 2–4 days, then rapidly disappeared, whereas the substance active against the other bacteria (penicillin) began to appear later. This is brought out in figures 5–6.

ISOLATION OF ANTIBIOTIC SUBSTANCES FROM FUNGI

Next to the isolation of antagonistic fungi, the problem of the nature and preparation of the active antibiotic substance produced by these organisms is of the greatest importance. Several cultures were selected for further study. They were found to produce substances highly active *in vitro* against a great many bacteria, largely the gram-positive and certain gram-negative forms.

Various solvents were used for the isolation of the antibiotic substances from several of the new fungi. The results obtained from only one organism, namely, *A. fumigatus* strain No. 84, is reported here. This organism was found to produce an active antibiotic substance which could easily be recovered from the medium by means of certain solvents and could be concentrated. This substance, tentatively designated as *fumigacin,* was found to be markedly different from penicillin.

The course of formation of this antibiotic substance is illustrated in figure 7. The substance was found to be soluble in ether, chloroform and alcohol, and partly soluble in water. It could be extracted from the medium by the first two reagents. Activated charcoal (norit) completely removed the substance from the medium; it could then be recovered from the norit by treatment with chloroform, or better still, by treatment with ether followed by chloroform. The following procedure was finally adopted for the isolation of this active substance:

Filtrate
(200 *B. mycoides* units and 3,000 *S. lutea* units per 1 ml.)

↓	↓
	Treated with norit ↓
Extracted with chloroform ↓	Extracted with ether ↓
	Extracted with chloroform ↓
Evaporated to dryness (yield, 120 mg. of crude material per 1 liter) ↓	Chloroform evaporated to dryness (yield, 100 mg. of material per 1 liter) ↓
Dissolved in alcohol ↓	Dissolved in alcohol (crystals produced on cooling) ↓
Activity on dry basis = 20,000 *B. mycoides* and *S. lutea* units.	Activity on dry basis = 6,000,000 *S. lutea* and 1,500,000 *B. mycoides* units.

The activity of crude fumigacin against different bacteria is illustrated in table 5. It is to be recalled that Raistrick (9) isolated a quinone, designated as "fumigatin" from *A. fumigatus.* This substance has recently been shown (7) to possess antibacterial activities. Quinones in general are known to possess marked antibacterial properties. Fumigacin is markedly different from

fumigatin both in chemical nature and biological properties, as will be shown in a subsequent paper.

The production of active substances by some of the other fungi isolated in this work has also been indicated.

TABLE 5

ACTIVITY OF CRUDE PREPARATION OF FUMIGACIN AGAINST VARIOUS BACTERIA

Test organism	Activity[1]
B. mycoides	1,500,000
B. subtilis	400,000
B. cereus	>200,000
B. megatherium	>200,000
B. brevis	>200,000
B. mesentericus	60,000
Micrococcus lysodeikticus	>200,000
Staphylococcus aureus	>200,000
Sarcina lutea	6,000,000
Brucella abortus	30,000
E. coli	3,000
Serratia marcescens	3,000
A. aerogenes	3,000
Ps. fluorescens	<2,000
Ps. aeruginosa	<2,000
Actinomyces 1	>20,000
Actinomyces 2	>20,000
Actinomyces 3	>20,000

[1] See table 1, footnote 2.

SUMMARY

Methods have been developed for the rapid isolation, from soils, manures, composts and other natural substrates, of fungi antagonistic to bacteria. No previous enrichment of the soil with bacteria is required.

A large number of antagonistic fungi were isolated by the use of these methods.

The antagonistic nature of the organisms was first established on solid media. The organisms were then grown in liquid media and the antibiotic activity of the culture filtrate was determined. Under the experimental conditions, some of the fungi produced such substances rapidly, whereas others showed only limited antibiotic activities.

The various antagonistic fungi thus isolated and tested were found to belong to a number of distinct taxonomic groups. So far,

nine groups have been recognized. Some of these groups belonging to the genus *Aspergillus* and the green *Penicillium* were particularly active; others, like the *Chaetomium* and some members of the *P. luteum* groups, had considerable activity; still others, such as members of the genera *Fusarium* and *Cephalosporium,* had very little activity. It is quite possible that the particular media used in these studies and the conditions of growth were not the most favorable for the production of the antibiotic substances by the last group of organisms. Active substances could readily be isolated from some of these fungi.

One of the most active fungi isolated in these studies was selected for detailed investigation, since this organism appeared to be different from all other antagonistic fungi thus far reported in the literature described above. The various strains of this fungus, belonging to the *A. fumigatus* group, differed considerably in their activity, but they all appeared to produce the same type of antibiotic substance. Methods were developed for the concentration and isolation of this substance. The organism is grown in a synthetic (glucose-nitrate) medium for 5–10 days; the filtrate is treated with norit, and the active substance removed from the latter with chloroform. The substance is soluble in chloroform and in alcohol, and partly soluble in ether and in water. It is thermolabile in the culture filtrate; however, when removed from the filtrate and concentrated, it becomes more thermostable.

This antibiotic substance, tentatively designated as *fumigacin,* possesses characteristic antibacterial properties, which are typical of the antibiotic group of substances as a whole, namely, that they are selective in their action. Much higher concentrations were required to inhibit the growth of *E. coli* and other gram-negative bacteria than of the gram-positive bacteria. There was, however, considerable variation in the degree of sensitivity of the gram-negative as well as of the gram-positive bacteria. This substance showed, in this respect, one interesting peculiarity, namely, that it was more active against *B. mycoides* than against *B. subtilis,* whereas other antibiotic substances, tested against these two organisms, showed the reverse effect.

APPENDIX

Medium 1. Bacterial agar.

Glucose	10 gm.
KH_2PO_4	1 gm.
Washed agar	15 gm.
Distilled water	1000 ml.

Heavy suspension of washed bacterial cells added to each tube of agar melted and cooled to 42° C.

Medium 2. Glucose-peptone or fungus agar.

Glucose	10 gm.
Peptone	5 gm.
KH_2PO_4	1.0 gm.
$MgSO_4.7H_2O$	0.5 gm.
Agar	15 gm.
Distilled water	1000 ml.

Medium 3. Glucose-nitrate or Czapek-Dox agar.

Glucose	40.0 gm.
$NaNO_3$	3.0 gm.
KH_2PO_4	1.0 gm.
$MgSO_4.7H_2O$	0.5 gm.
KCl	0.5 gm.
$FeSO_4.7H_2O$	0.01 gm.
Tap water	1000 ml.

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ZYGOSACCHAROMYCES ACIDIFACIENS: A NEW ACETIFYING YEAST

Walter J. Nickerson, Jr.[1]

(with 4 figures)

The organism to be described in this paper was isolated in the spring of 1938 from a bottle of domestic red wine turning sour. From wine souring to vinegar, one ordinarily expects to isolate bacteria belonging to the genus *Acetobacter;* however, careful examination both microscopically and by plating on nutrient agar failed to reveal members of this genus as present. On the contrary, a yeast was found in large numbers. So far as could be determined, the yeast was the sole occupant of the liquid in the bottle, thus confronting us with that very rare condition, namely, a pure culture in nature. This condition may have resulted, in part, from the high acidity of the wine for the pH was determined colorimetrically to be 2.2.

Single cell isolations of the organism were made using the Hansen technique; several isolates kept in culture for over a year showed the same properties and showed no variation in that time. The isolate kept in culture for the past four years, similarly, has shown no sectoring nor other variation.

The first attempts at identification of this yeast met with considerable difficulty. I tentatively considered the organism as belonging to the genus *Mycoderma* which includes imperfect yeasts, widespread in air, and frequently to be found in alcoholic solutions. They ordinarily form a scum on the surface of liquid media and rarely carry out an alcoholic fermentation, but are notorious for the production of acid from alcohol. *Mycoderma vini* (according to Lodder's 1934 classification) was the closest approach that could be made for this isolate but the position was actually untenable since no pellicle was ever formed by the yeast on any liquid medium and since the yeast did carry out an alcoholic fermentation.

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The situation regarding the taxonomy of this yeast was clarified by one of those fortunate accidents. A plate culture on nutrient-agar became contaminated with *Aspergillus niger* and examination of cells from this plate revealed many conjugating as iso-gametes and some conjugating pairs possessing spores with smooth walls. Such characteristics belong only to members of the genus *Zygosaccharomyces* since this is the only genus of budding yeasts to have conjugation of morphologically similar cells preceding the formation of spores with smooth walls. That the organism isolated was the organism seen conjugating (and not some other contaminant) was established by going back to pure cultures of the isolated organism; by counting several hundred cells it was found that there was indeed a small percentage conjugation in older cultures.

The effect of *A. niger* in causing a high percentage of the yeast cells to conjugate and the general problem of the chemical control of conjugation in the genus *Zygosaccharomyces* has been investigated (Nickerson & Thimann, 1941, 1942). Briefly, it may be stated that there are two extractable substances elaborated by the growth of the mold; these substances, one of an acid nature, in combination, provoke extraordinary increases in the percentage of cells conjugating. The action of the two components has been duplicated with riboflavin (vitamin B_2) and glutaric acid.

The genus *Zygosaccharomyces* Barker belonging to the family Saccharomycetaceae is a large one numbering about 60 reported species. Since the genus has never been monographed, some of the species may no longer be valid, but it is clear nevertheless that caution is necessary before establishing a new species amidst such a large and varied assemblage as already exists. Therefore, it is only because of the unusual property of this yeast in making large amounts of acid (as the proposed specific name "acidifaciens" indicates), that I am proposing this as a new species.[2] I have checked its properties against descriptions of all the species (over 60) to be found in the literature, and none is similar to it. Dr. E. M. Mrak of the University of California has very kindly checked my observations and agrees that it is apparently a new species.

[2] Cultures are being deposited with the American Type Culture Collection and with the Culture Collection of the Laboratory of Cryptogamic Botany, Harvard University.

MORPHOLOGICAL CHARACTERISTICS

Non-sexual phase.

Cells in malt extract broth (Difco) after 24 hours are ellipsoidal, 3–5 by 6–8 μ in size; mode of 100 counts was $4.3 \times 6.8\ \mu$. After three days the dimensions and shape are still the same. The vegetative cells are always single or in pairs; there is no tendency to form budding chains nor towards mycelial formation in any medium used. On wort agar after three days, cell size and dimensions are the same as in malt extract broth; figure 1 *a* shows the appearance of cells from a 3 day culture on wort agar. In old cultures cells frequently elongate but do not form chains. Budding is chiefly terminal or slightly sub-terminal. It has been pointed out (Nickerson, **1942**) that the bud of a yeast cell always arises so that its long axis is perpendicular to a tangent to the mother cell at the point of contact. The cells are vacuolated, colorless, and hyaline.

Ascosporic phase.

Production of ascospores in this species is preceded by conjugation of isogamous cells (FIG. 1 *b*). Usually only two spores are produced, one in each cell; however, four spores with two in each cell are occasionally seen. Sporulation occurs readily with the usual methods such as plaster blocks, Gorodkowa agar, etc., by the end of six days. The most favorable conditions for conjugation and resultant spore production are, however, on wort agar to which has been added a few cc. of the filtrate obtained from the growth of *Aspergillus niger* in malt extract broth. The ascospores formed are spherical, smooth, and 3–4.5 μ in diameter; the mode of counts on 100 spores was 3.5 μ.

Parthenogenesis is extremely rare in this species; a single cell possessing spores is practically never seen. While the conjugation has been called isogamous, an occasional case of anisogamy or conjugation between cells of noticeably different sizes has been observed; in this latter case, spores are formed only in the larger of the two cells. On staining water mounts of conjugating cells with methylene blue, it has been observed that in many cases (sometimes as high as 80 per cent of the conjugating pairs) one

of the cells of a pair takes up the stain while the other does not; a spore is generally present in each cell. This may possibly be an indication of physiological heterogamy. The production of more than one conjugation tube by a cell has occasionally been seen, as has the conjugation of one cell with two cells as a result of possessing more than one tube.

CULTURAL CHARACTERISTICS

In liquid culture, both in malt extract broth and in 10 per cent honey broth, there is no tendency towards formation of a pellicle, or of a yeast ring. The liquid remains clear during fermentation and CO_2 production is moderate.

Temperature limits for budding as determined by growth of slant cultures on agar are as follows: optimum, 26–28° C.; maximum 38–39° C.; minimum 4–5° C.

On a wort agar (Difco) streak, growth is good; both surface and margin are smooth with the growth spreading slightly. Lustre of the streak is dull and dry; the color is brownish-white; texture is soft and the form raised; no pseudo-mycelium is present. It is interesting that on wort agar of pH 4.7 the color is brownish-white (Ridgway, drab is the closest approximation), while on glucose-agar of pH 7.0 the color is cream-white (Ridgway, Tilleul-buff) and on glucose-chalk agar of pH 8.3 it is dead white (Ridgway, white).

The surface of the giant colony on 15 per cent honey agar is smooth with 2–3 lines radiating from the center (FIG. 2 *a*); this is a medium suggested by Lochhead (**1929**). On wort agar the surface of the young giant colony is somewhat punctate (FIG. 2 *b*) while a very old colony on the same medium shows the characteristic radiating lines, papillae, and a fringe of cells burrowing into the agar (FIG. 2 *c*).

A gelatin stab-culture shows no liquefaction even after three months; growth is best on top with no change in the medium.

PHYSIOLOGICAL CHARACTERISTICS

Glucose, fructose, and mannose are fermented strongly. The following are not fermented: galactose, sucrose, maltose, lactose, raffinose, arabinose, or dextrin. Growth with ethyl alcohol as the

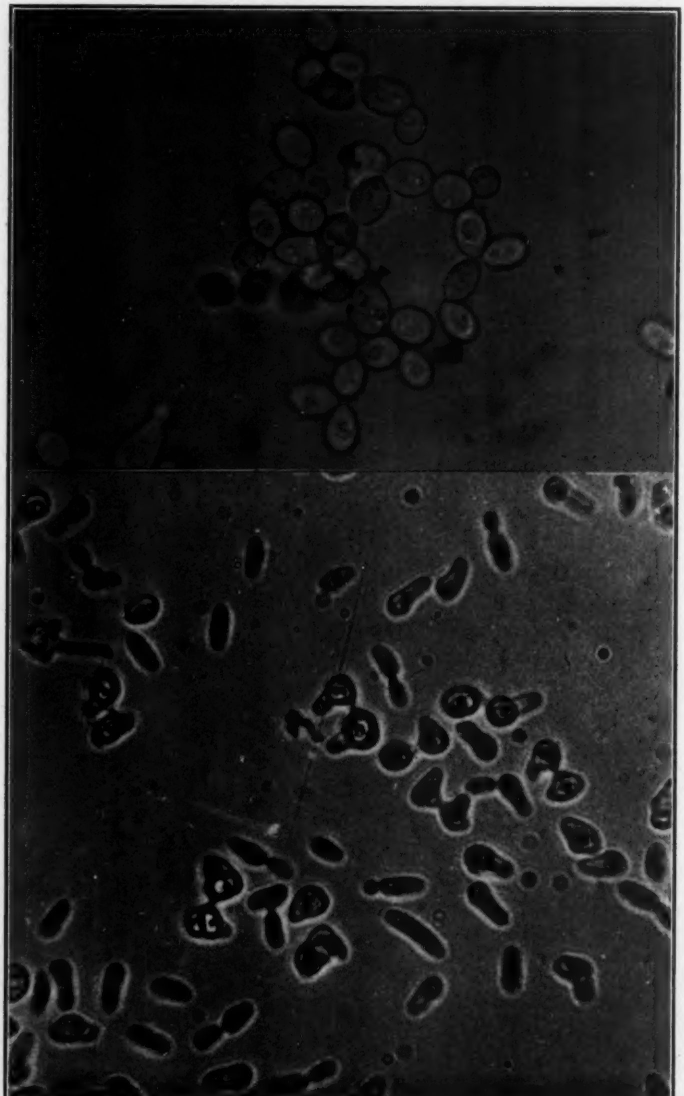

FIG. 1, above, vegetative cells of *Z. acidifaciens* from a 3 day culture on wort agar; below, conjugating cells of *Z. acidifaciens* with spores from a 9 day culture on wort agar treated with 5 mgm. glutaric acid and 100 micrograms riboflavin (total volume of plate culture 17 cc.).

sole source of carbon is light to moderate, no film being produced on the liquid. Growth with nitrate as the sole source of nitrogen (bios requirements [2] added with a trace of yeast extract) is moderate.

In respiration studies using the Warburg manometric technique it was found that the rate of oxygen consumption by this species was identical with glucose, ethanol, or acetic acid as substrate, but that fumarate, lactate, glutarate, and saccharic acid were not respired. The Qo_2 for glucose at 28° C. was 57, *i.e.*, 57 $mm.^3$ O_2 consumed per hour per mgm. dry weight of yeast.

The genus *Zygosaccharomyces* contains species exhibiting some of the most interesting properties. *Z. priorianus* Klöcker and *Z. rugosus* Lochhead & Farrell for example cause fermentation of 90 per cent glucose (a concentration of nearly 5 molar and one capable of producing an osmotic pressure of about 125 atmospheres!); *Z. guilliermondi* Dufrenoy grows in 28 per cent NaCl during the fermentation of citrons; Kroemer and Krumbholz (**1932**) have found some species capable of withstanding 40–50 per cent solutions of glycerin and saturated solutions of KNO_3. This species now adds production of 0.1 normal acid to the list.

Acid production.

This organism produces 0.10 normal total acid in a modified Williams' medium containing 30 per cent glucose. The composition of the medium is as follows:

* * * * * * *

KH_2PO_4	2.0 gms.	Asparagine	1.50 gms.
$(NH_4)_2SO_4$	3.0 gms.	Glucose (variable)	20.0 gms.
$CaCl_2$	0.25 gm.	Bacto-peptone	2.0 gms.
$MgSO_4$	0.25 gm.	Yeast extract	0.10 gm.

Distilled water to make 1 liter

* * * * * * *

Media containing various concentrations of glucose were inoculated and incubated for 15 days at 30° C. After this period the cultures were centrifuged and the clear supernatant liquid titrated with 0.10

[2] Dr. A. G. Lochhead, Central Experimental Farm, Ottawa, has very kindly determined that biotin and pantothenic acid are essential for this yeast, with thiamin overcoming an early lag in growth.

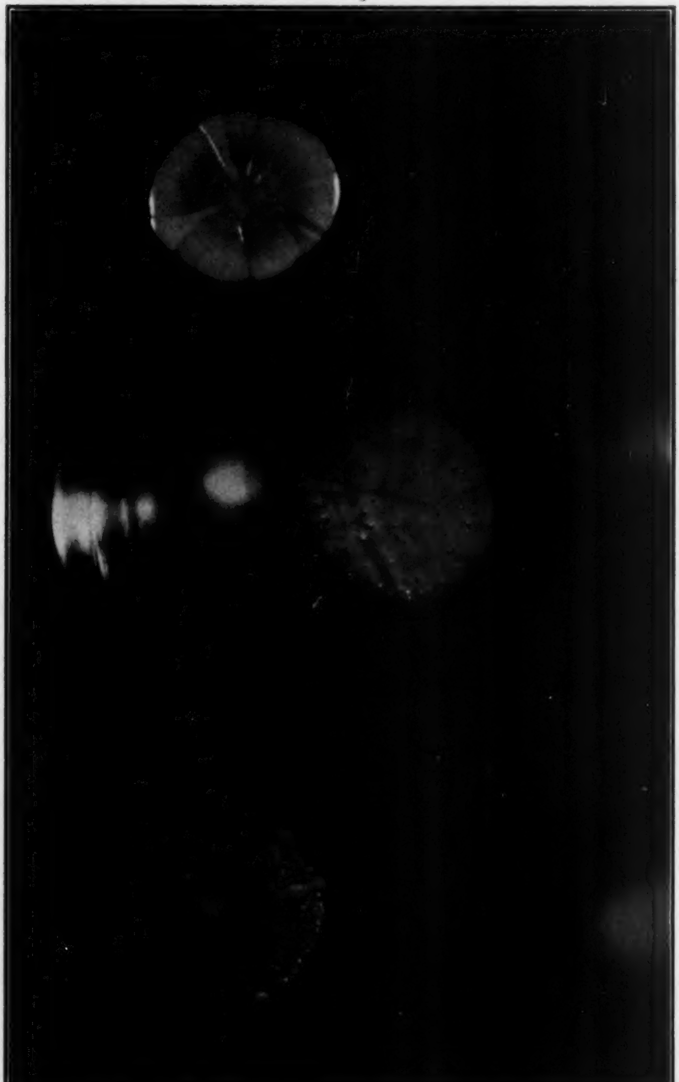

FIG. 2. Giant colonies of *Z. acidifaciens*. Above, 15 day colony on 15 per cent honey agar, actual diameter 15 mm.; center, 15 day colony on wort agar; actual diameter 20 mm.; below, very old colony, about 6 months, on wort agar, actual diameter 25 mm.

normal sodium hydroxide to determine the total acidity. Titration was to the first faint pink using 2 drops of 1 per cent phenolphthalein as indicator. The volatile acid was separated by steam

TABLE I

TOTAL ACID PRODUCTION WITH VARIOUS CONCENTRATIONS OF GLUCOSE; STERILE MEDIUM TOTAL ACID 0.018 N, pH 5.8; VOLUME OF SOLUTION 300 CC. IN 500 CC. FLASKS

Per cent glucose	Total acid	pH
2	0.028 N	5.6
5	0.046	4.3
8	0.049	4.2
10	0.052	4.1
15	0.064	4.0
20	0.066	4.0
25	0.094	3.9
30	0.101	3.8

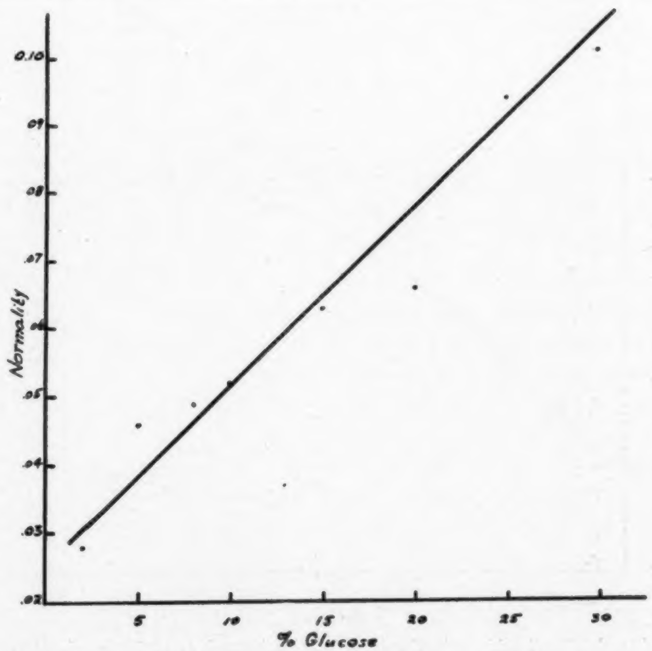

FIG. 3. Total acid production of *Z. acidifaciens* by fermentation of various concentrations of glucose in modified Williams' medium. Cultures 15 days old at time of sampling.

distillation and was found to comprise about 80 per cent of the total acid. Table I and figure 3 show the increase in total acid with increasing concentration of glucose. The volatile acid was found to consist almost entirely of acetic acid by means of the sharp melting point of the p-nitrobenzyl chloride derivative.

TABLE II

TOTAL ACID PRODUCTION WITH VARIOUS CONCENTRATIONS OF ETHANOL ADDED TO A MODIFIED WILLIAMS' MEDIUM CONTAINING 2 PER CENT GLUCOSE; STERILE MEDIUM TOTAL ACID 0.018 N, pH 5.8; VOLUME OF SOLUTION 300 CC. IN 500 CC. FLASKS

Per cent alcohol	Total acid	pH
2	0.029 N	5.1
5	0.033	4.9
8	0.039	4.2
10	0.040	4.1
12	0.041	4.1
15	0.040	4.1

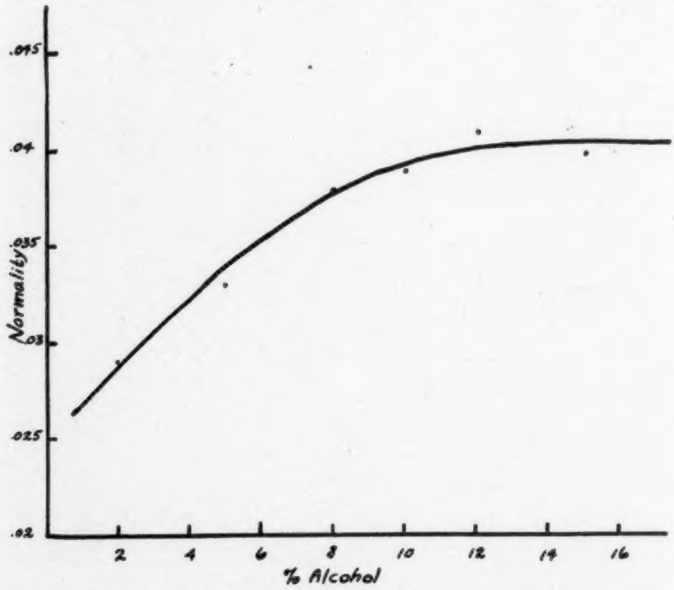

FIG. 4. Total acid production of *Z. acidifaciens* with added ethanol. Alcohol expressed as final concentration after addition to a modified Williams' medium containing 2 per cent glucose. Cultures 15 days old at time of sampling.

An experiment similar to the above with added ethyl alcohol was performed. The total acid produced was found to be 0.04 normal at a concentration of 10 per cent ethanol; while growth was good up to 15 per cent alcohol, no higher amounts of acid were produced as is clear from table II and figure 4. Volatile acid was not determined in this case. With neither the sugar series nor the alcohol series did the pH of the cultures fall below 3.8; this was probably due to the buffering action of the phosphate in the medium. The pH was measured using a quinhydrone electrode for all cultures at the time of sampling for acid.

A useful diagnostic test for acid production by yeast is the glucose-chalk agar plate as employed by Custers (**1940**) in his work on the yeast-genus *Brettanomyces.* The test is conducted as follows:

> A 5 per cent glucose-yeast extract agar with 0.5 per cent chalk (precipitated calcium carbonate) is autoclaved for 20 minutes at 15 pounds pressure; the agar is shaken well just before it hardens in a plate so as to disperse the chalk evenly. Plates are inoculated and incubated (preferably under both aerobic and anaerobic conditions) for 10 days at 25° C. Acid production is positive if nearly complete clearing of the agar occurs. A control plate inoculated with *Saccharomyces cerevisiae* should show little or no dissolution of the chalk.

With this test *Z. acidifaciens* shows some clearing aerobically but nearly complete clearing when grown anaerobically (in an evacuated desiccator over alkaline pyrogallol). More widespread incorporation of tests for acid production by yeasts into routine procedures might be of considerable use in the identification of this difficult group.

A more extended treatment of the physiology of this organism, including the phenomenon of acid production, will be presented elsewhere (Nickerson & Carroll, **1942**).

RELATION TO OTHER MEMBERS OF THE GENUS

Since this species will cause a fermentation of a 60 per cent glucose solution, but not of an 80 per cent solution, and since it produces rather large amounts of both alcohol and volatile acids from fermentation of a 30 per cent sugar solution, it is inter-

mediate between the two types of yeasts in this genus as found by Kroemer and Krumbholz (**1931**). Their groups were as follows:

I. High sugar tolerance, little production of volatile acid, vigorous fermentation of a 90 per cent glucose solution with little alcohol produced.
II. Less tolerant to high osmotic pressure, producing some volatile acid and alcohol.

This organism is certainly not so tolerant of high osmotic pressures as are two species described by Lochhead and Heron (**1929**), *Z. Nussbaumeri* and *Z. Richteri*. *Z. acidifaciens* is rather an osmotoduric organism. The distinctions between tolerance of sugar concentrations and demand for high concentrations are not always sharp; they may lie in part in the previous history of the cells since adaptation to high sugar seems clearly possible. Dr. Lochhead (**1941**) suggests that temperature of incubation plays a part here. The author has chosen to use the words "osmotoduric" (withstanding, but not demanding, relatively concentrated solutions of sugar, salts, etc.) and "osmotophilic" in preference to the more customary term "osmophilic," since the former words express more clearly the root "osmotic." This species is similar to the following members of the genus in fermenting only glucose, fructose, and mannose, and having an isogamic conjugation preceding spore formation; it can be easily distinguished from these as noted, however.

1. *Z. Bailii:* forms long budding chains in young cultures; cells large and elongate, frequently amoeboid-shaped. Fermentation feeble and giant colony on wort agar slow growing and small. Does not grow with ethanol alone.
2. *Z. dairensis:* also forms long budding chains. Ferments raffinose weakly, and does not grow with ethanol as a sole source of carbon.
3. *Z. mellis:* forms a yeast ring in liquid culture, giant colony on honey agar is rough, with a notched edge, and a well defined concentric ring around the center. Vegetative cells nearly spherical 4.5–5 microns.
4. *Z. Richteri:* forms a yeast ring in liquid culture and liquefies gelatin in eight weeks. Tendency for cells to adhere together in clumps of various sizes.

Zygosaccharomyces acidifaciens sp. nov.

Young cells ellipsoidal (3–5 × 6–8 μ), always single or in pairs, no budding chains; budding terminal or slightly sub-terminal. Optimum temperature for budding 26–28° C.; maximum 38–39° C.; minimum 4–5° C. No ring or pellicle formed in liquid media. Streak culture on wort agar brownish-white, texture soft, both margin and surface smooth; no pseudo-mycelium formed. Surface of giant colony on 15 per cent honey agar smooth with 2–3 lines radiating from center; on wort agar, the surface is somewhat punctate. Gelatin not liquefied after 3 months. Ascospores formed after an isogamic conjugation; usually one spore per ascus. Spore is spherical, smooth, average diameter 3.5 μ. Parthenogenesis very rare. Fermentation of glucose, fructose, and mannose only. Growth with ethanol as sole source of carbon is positive (though light). Nitrate assimilation positive. Volatile acid production high from a sugar medium; will dissolve carbonate in glucose-chalk agar plates when grown anaerobically.

Cellulae parvulae ovoideae (3–5 × 6–8 μ), semper singulae aut binae, gemmarum catenae nullae; gemmae extremo aut paene extremo. Calor optimus gemmandi 26–28° C.; calor maximus 39–39° C.; calor minimus 4–5° C. Fluentibus in mediis annulus membranaque non formantur. Cultura linearis in agaro maltato fusca-alba, textum molle, lēvīs et margo et superficies; pseudomycelium abest. Superficies coloniae ingentis in agaro mellito 15% lēvīs cum duo aut tres lineis radiantibus. In agaro maltato superficies non nihil puncta. Gelatinum non liquefitur. Ascospora isogamete post copulatione formantur; plerumque unum sporum quoque in asco. Sporum globosum lēveque ist, longitudo media lineae mediae 3.5 μ. Parthenogenesis infrequens. Fermentatio glucosii, fructosii, mannosii, aliorum nullorum. Auctus parvus, quoad ethanol fons carbonis sola ist. Assimilatio nitratium adest. Aliquantum acidi fugientis de saccharonibus formatur. In agaro cerevisiae glucosato cum carbonato calcico in condicione anaerobia carbonatis calcicus liquefitur.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the assistance given and the interest shown in the course of this work by Profs. K. V. Thimann and Wm. H. Weston, Jr. The author is deeply indebted to Dr. E. M. Mrak of the University of California for checking many of the characteristics of this proposed species; and thanks is due to Mr. R. S. Friedman of the Harvard Biological Laboratories for assistance with the photographic work.

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THE OCCURRENCE OF AMPHISPORES IN THE LEAF RUST OF BLUEGRASSES [1]

John R. Hardison

(with 2 figures)

Leaf rust of bluegrasses caused by *Puccinia Poae-sudeticae* (West.) Jorstad causes considerable reduction in forage and seed yields of economic species of *Poa* in northwestern United States. Fischer (**2**) reported the disease in epiphytotic form in Washington state. Sprague (**5**, **6**) has also reported its occurrence in the Northwest, and the writer (**3**, **4**) reported its development in Michigan.

During the summer of 1941 this species was found to be the only leaf rust occurring on bluegrasses in the writer's grass pathology nursery at the Botanical Garden of the University of Michigan. The fungus is readily recognized by the presence of abundant, capitate paraphyses in the uredia. This character assumes considerable taxonomic importance, since telia are rarely produced (**1**). In October, 1941 the uredia on plants of a single nursery row of *Poa pratensis* L. became dark brown in color, which was found to be due to the abundant development of amphispores in the uredia. Inasmuch as amphispores have not been previously reported for *P. Poae-sudeticae*, their morphology (fig. 1) is here described in detail.

Puccinia Poae-sudeticae Emend.

Amphispores 19–23 × 21–28 μ, the wall 1.7–2.6 μ, minutely echinulate, the pores 6, scattered, the pedicel generally persistent, colorless, once to twice the length of spore.

[1] Part of this study was made while the writer was a member of the Department of Botany of the University of Michigan.

The writer gratefully acknowledges indebtedness to Dr. E. B. Mains and Dr. George W. Fischer for advice about the disposition of these collections and to Dr. George Harrar for use of laboratory facilities of the Department of Plant Pathology of the State College of Washington.

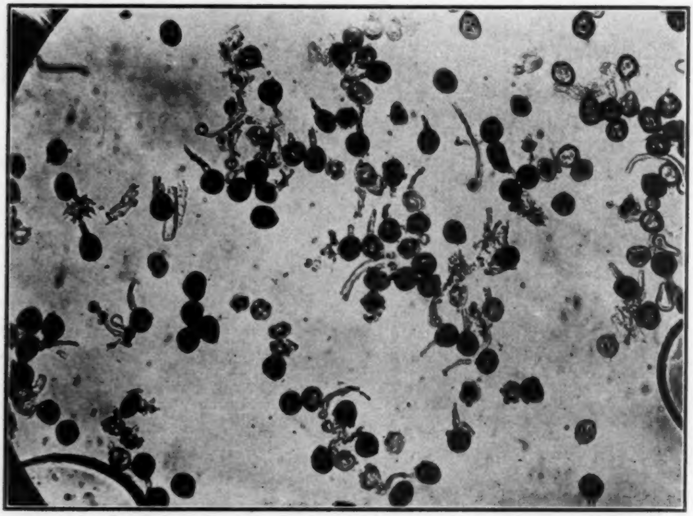

FIG. 1. Photomicrograph of *Puccinia Poae-sudeticae* showing amphispores and characteristic paraphyses, × about 200. (Photomicrograph by Dr. George W. Fischer.)

PURE CULTURE STUDIES

The amphispores would not germinate at maturity. A quantity of amphispores was placed outside October 29, 1941 in cheese cloth bags. On February 3, 1942 a two per cent germination was obtained and a 15–20 per cent germination on April 3. Germinating amphispores are shown in figure 2. Amphispores were sown on seedling plants resulting from seed of the same collection of *Poa pratensis* on which amphispores were collected. Uredia were produced seven days after inoculation, but no amphispores had developed 26 days after the inoculation. Urediospores which accompanied the amphisporic inoculum were non-viable. The uredia which developed from the inoculation with amphispores were characteristic in every respect for the species. The abundant capitate paraphyses accompanying the urediospores provide unmistakable evidence of the connection of the amphispores in the life history of *P. Poae-sudeticae*.

Since amphispores were formed in uredia on only one collection of *Poa pratensis,* the question naturally arises as to what conditions brought about their production. Other collections of this grass species in the same nursery were infected with uredia but were without amphispores. A large number of specimens of the rust on *P. pratensis* was collected throughout Washtenaw County,

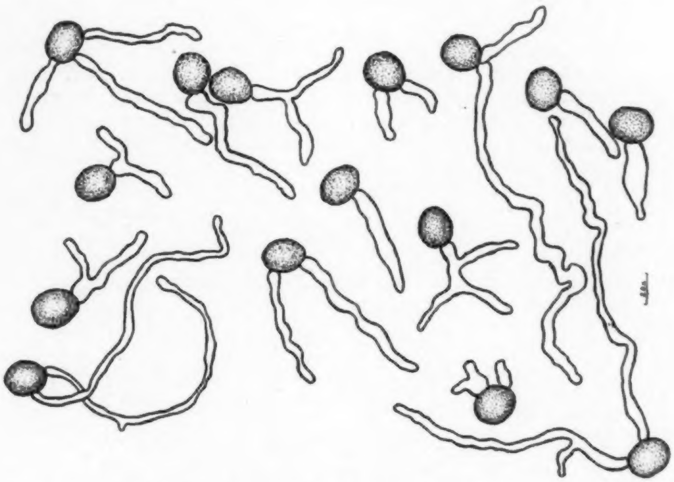

FIG. 2. Germinating amphispores of *Puccinia Poae-sudeticae* after 24 hours on two per cent plain agar.

Michigan, likewise with only uredia. The explanation of the appearance of amphispores remains a matter for conjecture, but it appears that their production must be related to the physiology of the host plant peculiar to the single grass collection.

Specimens have been deposited in the Mycological and Pathological collections of the Bureau of Plant Industry, Washington, D. C. and in the Herbarium of the University of Michigan.

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STUDIES IN SOME VENEZUELAN ASCOMYCETES COLLECTED BY C. E. CHARDON AND A. S. MULLER

Julian H. Miller and M. Gwendolyn Burton

(with 10 figures)

Some of the recent collections of these mycological explorers have been found to be most interesting links in the phylogeny of the Ascomycetes. They have provided additional evidence for the hypothesis of the origin of the complexly organized Pyrenomycetes and Discomycetes in the higher Myriangiales.

Besides this discussion, the writers have made a few taxonomic changes, and reduced to synonymy some of Dr. Chardon's genera and species, and described one new species.

Dothiora subtropica (Wint.) comb. nov. (figs. 1–2)

Blitrydium subtropicum Wint. Hedwigia **24**: 263. 1885.

Tryblidaria subtropica Rehm, Hedwigia **30**: 252. 1891.

Blitrydium subtropicum var. *microsperma* P. Henn. Hedwigia **41**: 304. 1902.

Protoscypha pulla H. Sydow, Ann. Myc. **23**: 403. 1925.

Myrianginella costaricensis Stev. Ill. Biol. Monog. **11**: 165. 1927.

Protoscypha subtropica Petrak, Ann. Myc. **32**: 363. 1934.

Pittierodothis Miconiae Chardon, Bol. Soc. Ven. Cien. Nat. **40**: 14. 1939.

This fungus is a parasite on species of *Bagnisiopsis* occurring on living leaves of various Melastomaceae in the tropics. An infected *Bagnisiopsis* stroma can be distinguished by the pulverulent, tobacco-brown surface, in contrast to the more or less smooth black unbroken surface of one not so parasitized.

A longitudinal section of such a parasitized stroma (figs. 1–2), shows a parallel layer of asci each in separate locules, surrounded

by very small-celled pseudoparenchyma. Below this one can often see the locules of the *Bagnisiopsis* with typical asci and spores. However, when a very young stroma becomes infected one is apt to think all of the tissue belongs to the *Dothiora*. The blending of the host and parasite is so complete that there is no perceptible line between the mycelia of the two fungi.

The ascospores of *D. subtropica* are large, broadly elliptical, muriform with slight constrictions at the middle septa, hyaline, $18–33 \times 8.5–13.5\ \mu$; while those of the *Bagnisiopsis Toledoi* Chardon, in this study, are broadly elliptical, one-celled, hyaline becoming brown late, $7.6–16 \times 7.6–11.4\ \mu$.

The illustration in figure 1 is from a mature *B. Toledoi* stroma which shows locules of the latter in other sections. The spiny projection at the left is typical of this *Bagnisiopsis* species. Figure 2, on the other hand shows a *Bagnisiopsis* stroma which became infected at a very early period.

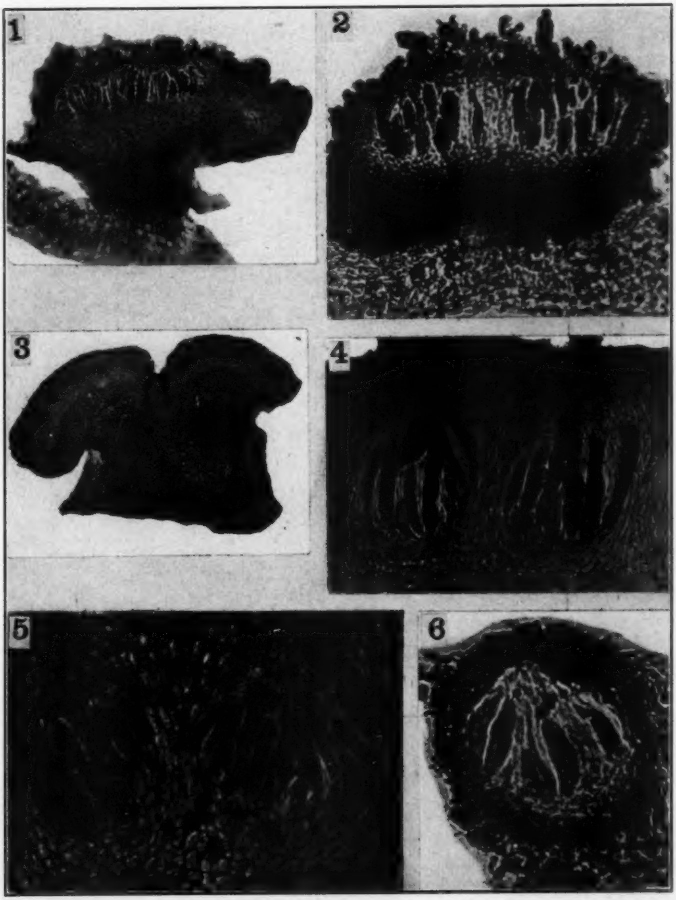
The striking resemblance to a Discomycete has resulted in its early position in genera of that group as shown in the above synonymy. Sydow (**10**) under *Protoscypha pulla,* also considered it a Discomycete, but observed its relationship to the Myriangiaceae. He failed to note the *Bagnisiopsis* connection. Later, however, he (**11**) recognized its parasitism and its identity with the Winter species, *subtropicum,* and with the Stevens species, *Myrianginella costaricensis.*

Petrak (**6**) cites the same synonymy and changes the name to *Protoscypha subtropica* and notes the *Bagnisiopsis* stroma.

Clements and Shear (**2**) place this fungus under *Protoscypha pulla,* in the Mollisiaceae with "apothecia folicole; epithecium present." Then on another page they also place *Protoscypha pulla* as a synonym under *Dothiora* Fries in the Myriangiaceae. They did not mention the growth on the *Bagnisiopsis* stroma.

Chardon (**1**) made a new genus and species, *Pittierodothis Miconiae,* combining the stroma of the *Bagnisiopsis* in his description. His flask-shaped locules apparently belong to *Bagnisiopsis,* but his spores are muriform, and so *D. subtropica.*

The type of *Dothiora* Fries is *D. pyrenophora* Fries, which equals *D. Sorbi* (Wahl.) Fuckel. European specimens studied by the writers on *Sorbus aucuparia,* collected in Latvia, showed black



FIGS. 1–2, *Dothiora subtropica* growing in stroma of *Bagnisiopsis Toledoi.* Photomicrograph of longitudinal section. 1, × 60. 2, section showing asci embedded in pseudoparenchyma, × 178. FIGS. 3–5, *Auerswaldia examinans.* Photomicrograph of longitudinal sections. 3, showing entire stroma with peripheral ascal locules, × 24. 4, showing two fascicles of asci in locules, surrounded by stromal pseudoparenchyma, × 200. 5, young asci expanding upward to form cavity, × 200. FIG. 6, *Mycosphaerella venezuelensis,* photomicrograph of longitudinal section, showing a single fascicle of asci in a stroma and remnants of stroma in centrum, × 394.

partially erumpent stromata on branches. The asci were immature, but arranged in a palisade layer in pseudoparenchyma. These generic characters, along with the muriform spores, definitely place the species *subtropica* in *Dothiora,* and *Protoscypha* Sydow and *Pittierodothis* Chardon become synonyms of that genus.

Dothiora is placed in the Discomycetes by Saccardo (**8**) and also Lindau (**5**). On the other hand, Theissen and Sydow (**15**), in an appendix to the Dothideales, describe *Dothiora* as showing relationships to the latter order. They say each ascus lies in a special locule and the ascal layer has the appearance of a Discomycete. Later they (**16**) place the genus in the Myriangiales, family Dothioraceae. Gäumann and Dodge (**3**) also have it in this position. Clements and Shear (**2**) have it in both the Phacidiaceae and in the Myriangiaceae.

The genus *Dothiora* represents a high level of development in the Myriangiales order in which the asci are no longer scattered at different levels, but are drawn into one layer, and are not globose or semiglobose but are elongate. The chief single character distinguishing the entire group is the inclosure of each ascus in pseudoparenchyma, with the concurrent lack of filiform paraphysoids or paraphyses. At maturity there is a dissolution of the stromal tissue above the asci and each swells, independently elongating upward to the surface, to discharge the spores.

There is no very definite term to characterize this type of fruiting body. It cannot be called an apothecium nor perithecium, and while cleistothecium is expressive it must cover such widely diverse shapes as pulvinate irregular masses, or minute or large globose, or even plainly disc-shaped ascocarps.

Dothiora subtropica has been found on several different Melastomaceae hosts as well as *Bagnisiopsis* species. However, in most cases the latter have not been determined. Winter and Henning cited it from Brazil only on Melastomaceae leaves, while Sydow (**10**) has it on *Miconia Thomasiana* from Costa Rica, and later he (**11**) has the fungus on *Miconia ambigua,* on Melastomaceae undet., *Clidemia* sp., and *Clidemia plumosa,* all in Venezuela. These were on *Bagnisiopsis* spp. and on *Sucinaria minuta.* Chardon specimen No. *2666* also from Venezuela is on *Miconia dodecandra* on *Bagnisiopsis Toledoi* and Nos. *3110* and *3749* are on

Miconia sp. The last is parasitizing *B. Toledoi*. The genus *Sucinaria,* mentioned above is a synonym of *Bagnisiopsis*.

AUERSWALDIA EXAMINANS (Mont. & Berk.) Sacc. Syll. Fung. **2**: 626. 1883. (FIGS. 3–5)

Sphaeria examinans Mont. & Berk. Hook. Lond. Jour. Bot. **1**: 156. 1842.

Dothidea examinans Mont. & Berk. Lond. Jour. Bot. **3**: 520. 1844.

Auerswaldiella disciformis Chardon, Bol. Soc. Ven. Cien. Nat. **40**: 11. 1939.

This species, anatomically, is a typical member of the Dothideales in the sense of the Friesian genus *Dothidea,* with its type *D. Sambuci* Pers. ex Fries. *Auerswaldia* differs from *Dothidea* chiefly in the possession of continuous instead of one-septate spores. The asci arise from convex masses of fertile hyphae, more or less evenly spaced in the periphery of the stroma (FIG. 3). In developing they expand vertically, producing a dissolution of the surrounding stromal elements (FIGS. 4–5). This creates a locule containing a fascicle of many asci inclosed by no differentiated wall, but only stromal pseudoparenchyma. There are no filaments between the mature asci in any one locule.

The locule pore is not a true ostiole in the sense of the one formed in the Sphaeriales. The tissue directly above the cavity, as shown in figure 5, is composed of very fine deeply staining pseudoparenchyma, contrasting rather sharply with the surrounding larger elements. This disintegrates as the asci mature, leaving an opening to the surface, which is not bordered by a specialized wall.

The type of *Auerswaldia examinans* was collected in Java. Later Rehm (**7**) described it from the Philippines and now Chardon and Muller have found specimens, Nos. *2411, 2556, 2404, 3295,* in Venezuela. No host has been cited in any case, only its occurrence on dead wood.

The Chardon specimens have been compared with part of the Montagne and Berkeley type in the Harvard herbarium. The latter shows the same dothideaceous locules and one-celled brown

ascospores, 15–22.8 × 9–11 μ. With a faint light, longitudinal striations were observed in the spore walls of both the type and those from Venezuela. This character has apparently been overlooked in previous descriptions.

Chardon thought there were paraphyses between the asci which accounts for his inclusion of the fungus in *Auerswaldiella.*

Auerswaldia examinans differs from *D. subtropica* in possessing a fascicle of asci in each cavity, instead of the single ascal locules, and in having one common pore for a group of asci to discharge their spores through, rather than each ascus attaining the surface through a separate opening. These constitute the chief differences between the Myriangiales and Dothideales concepts. Neither have specialized walls surrounding groups of asci nor filamentous threads in the centrum. The Dothideales could have arisen from the Myriangiales by the simple proliferation of the hypha producing the single ascus to form a fascicle.

Mycosphaerella venezuelensis sp. nov. (FIG. 6)

Maculae amphigenae, irregulariter orbicularae, pallide brunneae, centra interdum resolventia et cadentia, .5–3 cm. in diam., in vivis foliis; perithecia sparsa, epiphylla, minuta, globosa vel depresso-globosa, 75–100 μ in diam., atro-brunnea, in mesophyllo immersa, partietibus carbonaceis, 10–20 μ crassis, pseudoparenchymatis, ostiolo punctiformo; asci fasciculati, ex basi perithecii evoluti, cylindraceo-clavati, membrano .6–1.5 μ crasso, breviter stipitati, 45–50 × 11–12 μ; ascosporae 8, biseriatae, vel inordinatae, hyalinae, fusoido-ellipticae, utrinque obtusae, constrictae, 1-septatae, 16–19 × 4–6 μ; paraphyses nullae.

Spots amphigenous, irregularly orbicular, pale brown, centers breaking up and falling out, .5–3 cm. in diam., in living leaves; perithecia sparse, epiphyllous, minute, globose or depressed-globose, 75–100 μ in diam., dark brown, immersed in the mesophyll, with carbonous walls, 10–20 μ thick, pseudoparenchymatous, with punctiform ostiole; asci fasciculate, arising from the base of the perithecium, cylindric-clavate, with walls .6–1.5 μ thick, briefly stalked, 45–50 × 11–12 μ; ascospores 8, biseriate or inordinate, hyaline, fusoid-elliptic, with slightly obtuse ends, constricted, 1-septate, 16–19 × 4–6 μ; no paraphyses.

On living leaves of *Canavalia ensiformis,* Caracas, Venezuela, Muller No. *2368,* in Cornell Herbarium.

This fungus differs from the Philippine *Mycosphaerella Canavaliae* Sydow, in possession of larger spores. Sydow (**9**) gives those of the latter as 13–15 $\times$ 2–3 μ.

The genus *Mycosphaerella* as shown in figure 6 has all of the centrum characters of the locule of *Auerswaldia* (FIGS. 4–5). There are no paraphyses nor paraphysoids, only cellular remnants due to the dissolution and compression effects of expanding asci. *Mycosphaerella* then is apparently the high point in a developmental series beginning in the higher Myriangiales, and continuing through the compound Dothideales to the single stromal locules. We start with many locules, but only one ascus in each in *Dothiora,* then go to many locules, but several to many asci in a fascicle in each in *Auerswaldia,* and end the series with perithecial-like stromata containing only one locule with a more constant and compact fascicle in *Mycosphaerella.*

Epiphyma nervisequens (Chardon) comb. nov. (FIG. 7)

Dimeriellina nervisequens Chardon, Bol. Soc. Ven. Cien. Nat. **40**: 5. 1939.

This fungus is a parasite on leaves of *Lantana camara* in Venezuela. The ascocarps appear to the eye as single, superficial perithecia, in rows following the veins on the upper side of the leaves. Figure 7 is a picture of a longitudinal section of No. *2611,* the Chardon type. This shows plainly that the so-called perithecia are locules in an elongate stroma with only the upper part of the locule wall partially free. The stroma may contain single locules or many in a row. The separations between locules are entirely stromatic as in the Dothideales. The stromata are not completely free on the surface, but extend into a hypostroma in the upper mesophyll. There are filiform threads, or paraphysoids between the asci, connected above and below. The ascospores are one-celled, hyaline, elliptical, 19–25 $\times$ 7–9.5 μ.

The genus *Epiphyma* is based on *E. anceps* (Höhnel) Theissen, or *Botryosphaeria anceps* Höhnel. The writers were not able to obtain a specimen of the type to study. The description and illustration by Theissen (**12**) consists of free perithecial-like stromata on thin twigs, with no true ostiole, with asci surrounded by no

genuine paraphyses, but threadlike plectenchyma, with one celled hyaline ascospores. Later, he (**13**) says *Epiphyma* is a *Parodiella* with colorless, one-celled spores. Then in his (**14**) introduction to the Pseudosphaeriales he places it in that order and in the family Parodiellaceae.

Clements and Shear (**2**) have *Epiphyma* in the Sphaeriaceae—hyalosporae, with—"perithecia superificial from the first, not beaked, glabrous, and paraphysoids present."

Chardon (**1**) erected the genus *Dimeriellina* for this single species with approximately the same characters as given by Theissen, or Clements and Shear, for *Epiphyma.* However, he called the interthecial threads paraphyses instead of paraphysoids, and then he places his genus in the Perisporiales. The type is No. *2611* collected by Chardon and Barnes. Chardon cites another specimen, Muller No. *1941,* not studied by the writers.

Theissen and Sydow (**16**) separate the Perisporiales by closed Ascomycetes with globose perithecia without ostioles, which contain a free fascicle of asci arising from the base. Clements and Shear (**2**) distinguish the order on perithecia with ostiole and paraphyses usually lacking. *Dimeriellina* then with its paraphysoids and asci not in a fascicle cannot remain in the Perisporiales, but falls in the Pseudosphaeriales as a synonym of *Epiphyma* along with such genera as *Parodiella* and *Apiosporina.*

There is a species of *Epiphyma, E. neurophilum* Theiss. whose description fits the Chardon specimen very well, but it is on *Tibouchina* sp. from Colombia. This was not in the Theissen herbarium at Harvard, and so could not be studied. However, this may be a different species as the hosts are not the same.

Hypospila Oyedaeae (Sydow) comb. nov. (FIG. 8)

Phyllocelis Oyedacae Sydow Ann. Myc. **23**: 353. 1925.

Phomatospora Oyedaeae Chardon, Bol. Soc. Ven. Cien. Nat. **40**: 27. 1939.

The perithecia, usually separate, occasionally two or more together, are sunken in the mesophyll of the leaf, with true pseudoprosenchymatous walls, with a short broadly-papillate ostiole erumpent through a clypeus in the lower epidermis. The centrum

consists of a wall layer of asci interspersed with band-like paraphyses with free apices. At maturity the asci separate from their attachment, and the paraphyses gelatinize and disappear, leaving the cavity filled with free asci. The spores are spindle-form, fusoid, straight to slightly curved, 2–4 septate, hyaline, 18–32 × 6–8 μ.

There is a conidial stage in the Chardon specimens directly over each perithecium. This has apparently not been adequately described, although Chardon noted the presence of conidia and gave measurements. The writers did not see the Sydow specimen, but his description for the perithecial form fits the Chardon fungus exactly, and so it is logical to think he must have had the acervuli on his leaves. In every case sectioned there are acervuli opening on the upper side, and perithecial ostioles erumpent through the lower epidermis.

Conidial stage: Acervuli in spots developing under the upper epidermis, 200–300 μ in diam., orbicular to irregular, opening by large pore; conidiophores in palisade layer, with conidia filiform, curved, hyaline, 15–25 × .5–1 μ.

The connection between the acervulus and the perithecium has not been established by inoculations, but the relationship seems obvious as the two are together in every spot.

On *Oyedaea verbesinoides.* The Sydow type is from Costa Rica, while the Chardon collections, Nos. *2509, 2526, 2716, 2874,* are from Venezuela.

Sydow created the genus *Phyllocelis* for this fungus and characterized it as diaporthoid. The characters of true perithecia with asci that float out in masses in water are typical of the family Diaporthaceae. Chardon evidently failed to note this fact, and also that the ascospores are septate, and so he places the species in *Phomatospora,* describing it as new.

Clements and Shear (2) have *Phyllocelis* in the Hypocreaceae hyalophragmiae, with "perithecia in a stroma, not compound, ostiole broad-conic erumpent; folicole." There is very little fungous tissue outside of the perithecium that could be called a stroma and the clypeus is not very well developed. The fungus does not appear colored to the writers, so it could hardly fall in the Hypocreaceae.

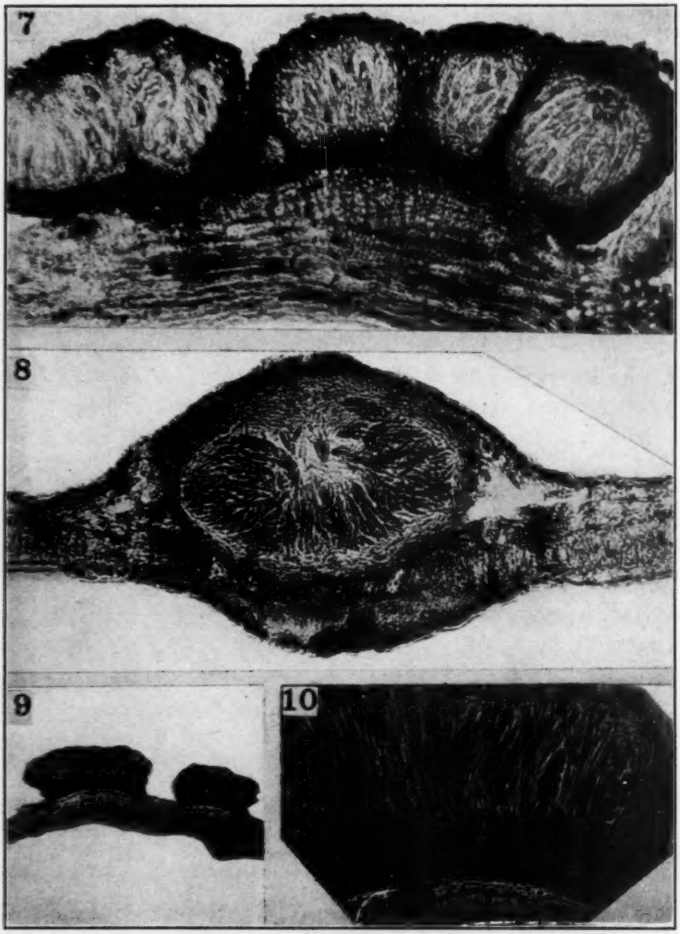


FIG. 7, *Ephiphyma nervisequens.* Photomicrograph of longitudinal section showing several locules in each stroma with hypostroma in leaf, × 94. FIG. 8, *Hypospila Oyedaeae.* Photomicrograph of longitudinal section, showing distinct wall to perithecium inclosing diaporthoid asci and paraphyses; and the conidial stage, acervulus, opening on the lower surface, × 150. FIGS. 9–10, *Mollisia Grisleae.* Photomicrograph of longitudinal sections. 9, showing two entire apothecia, × 75. 10, part of apothecium enlarged, showing asci embedded in paraphyses and the dark pseudoparenchymatous exciple, × 338.

The genus *Hypospila* Fries is based on *H. pustula* (Pers. ex Fries) Karst. according to Clements and Shear (**2**). They place this genus in the Sphaeriaceae-hyalophragmiae, with "perithecia innate, with clypeus, not beaked, and paraphyses lacking."

Von Höhnel (**4**) finds *Sphaeria pustula* Pers. is not the type of *Hypospila* Fries, and creates a new genus for it—*Chalcosphaeria.* His conception of the latter is, perithecial nucleus diaporthoid, with hyaline, spindleform spores chiefly 2-celled, but occasionally 3–4-celled, on leaves. It seems best in cases like this to arbitrarily pick a species for the type that is in line with the current concept of the genus, rather than go back and dig up the first described species which would result in much present confusion. In following Clements and Shear then in adopting *S. pustula* for the type the concept of *Hypospila* remains the same as for the last 70 years. The characters of *Phyllocelis* are identical with this characterization of *Hypospila,* and it is reduced to synonymy.

Hypospila Oyedaeae represents a high level of development in the Ascomycetes. With its diaporthoid centrum inclosed by a special wall it is quite distinct from the Pseudosphaeriales form, *Epiphyma nervisequens,* or the Dothideales branch with the examples of *Auerswaldia examinans* and *Mycosphaerella venezuelensis.* Both groups must have developed from the more primitive Myriangiales.

Mollisia Grisleae (Sydow) comb. nov. (FIGS. 9–10)

Antimanoa Grisleae Sydow, Ann. Myc. **28**: 170. 1930.

This minute leaf parasite forms discs on the upper surface, 150 to 300 μ in diam. When wet they expand showing a concave to flat surface with thick-walled pseudoparenchymatous dark exciple. The ascospores are one-celled, hyaline, elliptical, 13–17 $\times$ 5–7 μ. The paraphyses are filiform and slightly branched. Figure 10 shows the large-celled exciple and hypothecium resting on the leaf epidermis.

Both the Sydow and Muller (No. *1942*) collections are from Venezuela on leaves of *Grislea secunda.*

Sydow created the genus *Antimanoa* for this species, and says it has plain Hemisphaeriales relationships, and also corresponds to a superficial Phacidiaceae. When dry and shriveled the apothecia do become flattened-hemispherically in a manner suggesting the former order, but when wet they expand into a typical *Mollisia,* with disc with even border and dark exciple.

If the *Dothiora* in figure 2 is compared with that of this *Mollisia* in figures 9 or 10 a striking resemblance is to be seen. The chief difference lies in the cellular interascal tissue in the former and free filiform threads in the latter. The basal tissue is dark pseudoparenchymatous in both cases. Such Discomycetes could have arisen from the higher Myriangiales by the development of threads between the asci to replace the cellular interthecial tissues.

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A DASYSCYPHA FOLLOWING CRONARTIUM RIBICOLA ON PINUS MONTICOLA.[1] I

RICHARD T. BINGHAM [2] AND JOHN EHRLICH [3]

(WITH 2 FIGURES)

During the course of a study on secondary fungi associated with blister rust cankers of *Pinus monticola* Dougl. a *Dasyscypha* of uncertain identity was frequently encountered. This fungus had in the past been variously designated as *D. Agassizii* (Berk. & Curt.) Sacc. and *D. calyciformis* (Willd. ex Fries) Rehm, apparently with some question as to its identity. In view of the confusion which has existed between these fungi, all three have been studied.

DASYSCYPHA CALYCIFORMIS

Late in the eighteenth century, Willdenow (**1787**: 404) described a fungus which he called *Peziza calyciformis* and, without mentioning any host, reported that it occurred on tree trunks and on rotting twigs on the ground ("In arborum truncis, ramulisve dejectis putrescentibus"). He gave neither ascospore measurements nor other means by which this fungus might be recognized today. Willdenow based his description on Batsch (**1786**: 195), who was preceded in the use of the specific epithet *calyciformis* (according to Plassmann (**1927**: 8)) by Gleditsch (**1753**), who, in turn, cited Dillenius (**1719**: 195) as the presumed author of the epithet.

Later, however, in the Systema, Fries (**1822**: 91–92) described three forms of a fungus which he called *P. calycina,* the first of

[1] Help in the study and identification of *Dasyscyphae* has been given by Dr. Glenn Gardner Hahn. Dr. Fred J. Seaver, Mr. John A. Stevenson, Miss Edith K. Cash, Dr. J. M. Greenman, Dr. David H. Linder, Dr. Walter H. Snell, Dr. E. B. Mains, Dr. Sanford M. Zeller, and Mr. C. R. Stillinger all aided by lending herbarium specimens.

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which, "α. *Pini silvestris,*" he reported as synonymous with *P. calyciformis* Willd. and *P. calycina* Schum.[4] In so doing Fries lowered the fungus under consideration to varietal status. Finally Rehm (**1893**: 834–835) again raised this fungus to specific rank and applied to it the epithet *calyciformis* of Willdenow, which Fries had recognized in the Systema. Since *calyciformis* is the first legally published epithet for this species, Rehm thus acted in conformity with the International Rules (Briquet **1935**: Art. 58). At the same time Rehm transferred the fungus to the genus *Dasyscypha* and published the new combination *D. calyciformis.* According to Recommendation XXXII, the species should, therefore, be cited as *Dasyscypha calyciformis* (Willd. ex Fries) Rehm.[5]

Opportunity has been afforded for the examination of an authentic Rehm exsiccatus (Ascomyceten *1163*), issued in 1896 under the name of *D. calyciformis* Willd. This exsiccatus was acquired by the New York Botanical Garden Herbarium (NY.[6]) as a part of the Ellis Collection and was made available for study through the courtesy of Dr. Seaver. Several well preserved apothecia are present, the morphological details of the perfect stage agreeing closely with those in Rehm's description.

[4] Fries probably confused two distinct species, for Schumacher (**1803**: 424) (according to Hahn and Ayers (**1934**: 76)) had described his Discomycete as occurring on cones of (?) *Picea abies* Karst. ("in strobylo *Pini abietis*"). It seems unlikely that Schumacher's fungus on spruce cones was the same as Willdenow's fungus on trunks and twigs, especially since none of the specimens or reports examined in the course of the present study show Willdenow's fungus to occur on any substratum other than the bark of *Abies, Picea,* or *Pinus.* On the other hand, it is not unlikely that Fries may have been correct in his belief that Willdenow's *P. calyciformis* was synonymous with his own *P. calycina α. Pini silvestris* since *D. calyciformis* presumably does occur on bark of *Pinus sylvestris* L. In any event, Fries recognized and published the epithet *calyciformis* of Willdenow.

[5] Since Hahn & Ayers (**1934**: 77) have shown that the name *D. calycina* should be reserved for a large-spored species of Fuckel (**1869/70**: 305), Plassmann's (**1927**: 8–9) use of this name for *D. calyciformis* is regarded as untenable.

[6] Herbarium abbreviations follow Lanjouw (**1939**).

FIG. 1. *Dasyscypha calyciformis* (Willd. ex Fries) Rehm. Drawings made with aid of a micro-projector; those of the perfect stage from Rehm: Ascomyceten *1163,* those of the imperfect stage from Kryptogamae Exsiccatae *1821.* *A,* asci and ascospores; *B,* ascospores; *C,* excipular hairs; *D,* conidiophores and phialides; *E,* conidia.

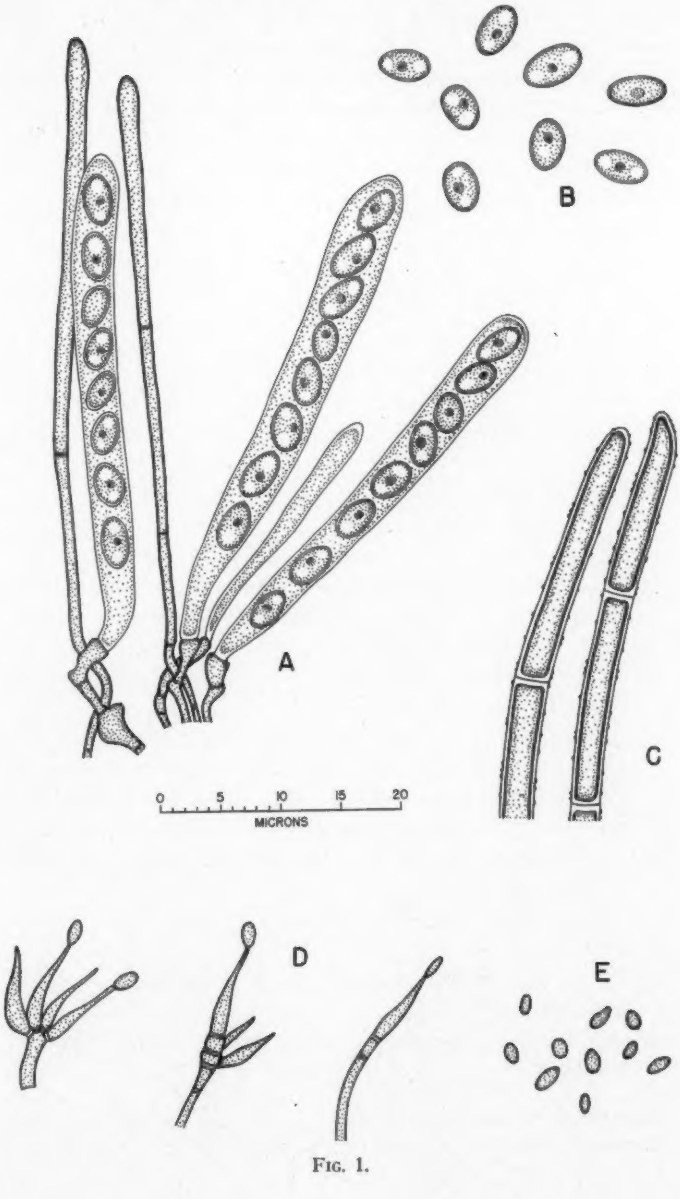

FIG. 1.

An amended description of *D. calyciformis* follows, using this exsiccatus as its base. It is recognized that this description is inadequate because it was not possible to study the fungus in culture or to examine fresh European material on various hosts. Only a limited number of dried specimens, not including any types, were available. It is intended that this description will, nevertheless, serve to distinguish this species from the fungus on *Pinus monticola*.

DASYSCYPHA CALYCIFORMIS (Willd. ex Fries) Rehm in Rab. Krypt.-Fl. **1** (3): 834–835. 1893.

Elvella calyciformis Batsch, Elench. Fung., Cont. **1**: 195. 1786. —Teste Hiley, Fungal diseases of the common larch: 76. 1919.

Peziza calyciformis Willd., Fl. Berol. Prodr.: 404. 1787.—Teste Fries, Syst. Myc. **2**: 91. 1822; Rehm, *loc. cit.*

Octospora calyciformis Hedw., Descr. Musc. Frond. **2**: 64. 1789.—Teste Rehm, *loc. cit.*

Peziza calycina α. *Pini silvestris* Fries, Syst. Myc. **2**: 91. 1822.

(?) *Dasyscypha calycina* (Schum.) Fuckel, Symb. Myc. 305. 1869/70.—Teste Oudemans, Enum. Syst. Fung. **1**: 367. 1919. Cf. Hahn in Mycologia **26**: 76–77. 1934.

Helotium calycinum Karst., Myc. Fenn. **1**: 154. 1871.—Teste Rehm, *loc. cit.*

(?) *Dasyscypha bruyeriensis* (Roum.) Sacc. in Michelia **2**: 330. 1881.—Teste Rehm, *loc. cit.* Sed. syn. *D. subtilissima* (Cooke) Sacc., Syll. Fung. **8**: 438. 1889;—teste Sacc., *loc. cit.*

Lachnella calycina Karst., Rev. Monogr. Ascom. **2**: 131. 1885. —Teste Rehm, *loc. cit.*

Erinella calycina Quél., Enchir. Fung. Europa: 303. 1886.—Teste Rehm, *loc. cit.*

Helotium calyciforme Wettstein in Bot. Centralbl. **31**: 285. 1887.—Teste Rehm, *loc. cit.*

Dasyscypha calycina Fries sensu Plassmann, Untersuchungen über den Lärchenkrebs: 8–9. 1927.

Apothecia abundant, sub-phellar or shallowly intraphellodermal, erumpent, gregarious or solitary, occasionally joined at the base on

a subicle; *stipes* short but distinct, each with one to several cups; *cups* when young globular, closed, with age opening circularly and expanding under moist conditions to a cup-like or saucer-like form, margins at maturity moderately thin, externally whitish with excipular hairs; *hairs* long, unbranched, cylindrical, moderately to considerably roughened by minute tubercles, the tips usually subacute, occasionally rounded, extremities ordinarily unswollen, noticeably septate at intervals of 10 to 25 μ, 2.5–4.0 μ broad, persistent except in the oldest and most weathered specimens (FIG. 1, *C*); *discs* orange to orange-yellow, small, 0.5–2.0 mm. broad; *hyphae* of the context variable in width, 2.0–5.0 μ broad, septate, profusely branched. **Asci** cylindrical to cylindric-clavate, with rounded apices; size range (90) 36.0–58.5 × 4.0–6.0 μ, commonly 43–54 × 4.5–5.5 μ (FIG. 1, *A*). **Ascospores** eight; *arrangement* uniseriate, oblique with the basal spores vertical, occasionally irregular or biseriate; enclosing *membrane* rounded at the apex, following the contour of the ascus wall; *shape* elliptical to plump-ovate, occasionally with one end (the lower when in the ascus) slightly more acute or, in older and more weathered specimens, collapsed making the shape irregular; *walls* thin; *contents* hyaline, indistinctly nucleate and biguttulate, continuous while in the ascus (germination not observed); *size* range (165) 4.0–7.5 × 1.5–3.5 μ, commonly 4.5–7.0 × 2.0–3.0 μ (FIG. 1, *A* & *B*). **Paraphyses** usually exceeding the asci by from 5 to 10 μ, relatively sparse, relatively coarse and stout for their length, occasionally very minutely roughened, branching not seen; *shape* cylindrical or tapering slightly and evenly to the base, tips rounded or sub-acute with obtuse extremities; *contents* hyaline, obscurely septate, minutely guttulate; *size* range (70) 45.0–77.0 × 1.0–3.0 μ, commonly 55–65 × 1.5–2.5 μ (FIG. 1, *A*).

Imperfect fruiting bodies infrequently found, inconspicuous, developing from light-colored, sub-phellar, erumpent stromata; with age becoming multiloculate and the outer wall and overlying bark more or less completely lost. **Conidiophores** erect, entirely lining the locules; simple or sparsely branched, noticeably septate, hyaline, minutely guttulate; bearing sparsely at the sides and sparsely or profusely at the apices the unicellular, curved, subulate spore-bearing elements (phialides [7]). **Conidia** abstricted from the acute tips of the phialides, hyaline, continuous (germination not observed), very thin-walled, usually long-elliptical to ovate, occasionally allantoid, size range 2.5–4.0 × 0.5–1.5 μ (FIG. 1, *D* & *E*).

[7] After Mason (**1937**: 86–89).

TYPE SPECIMEN

The existence of a type specimen has not been reported and no effort was made to locate one in the course of the present study.

SPECIMENS EXAMINED

Exsiccati apparently correctly designated as *D. calyciformis:* Rehm, Ascomyceten *1163* (1896), "*Dasyscypha calyciformis* (Willd.)" (NY.); containing twenty or more well preserved apothecia and no imperfect fruiting bodies. A tabular summary, showing the similarity between the perfect stage of this exsiccatus, the perfect stage of the fungus as described by Rehm (**1893**: 834–835), and the perfect stage of the fungus as described by Jørstad (**1925**: 39), Schellenberg (**1905**: 270), and Maublanc (**1904**: 234), follows.

	Asci	Ascospores	Paraphyses	Hairs
Ascomyceten *1163*	43–51.5 ×4–6 μ	4–7×2–3.5 μ	49.5–71.5 ×1.5–2.5 μ	3 μ wide
Rehm's description	50–60 ×4.5–5 μ	5–7×2.5–3 μ	1–2 μ wide	3.5–4 μ wide
Jørstad's description	—	5–7.5 ×2–3 μ	—	—
Schellenberg's description	50–60 ×4–7 μ	5–8×2–3 μ	50–60 μ long	—
Maublanc's description	40–50 ×4–4.5 μ	6–8×2.5–3 μ	—	—

It can be seen that the exsiccatus fits Rehm's description quite closely in these characters of the perfect stage. More complete examination of the exsiccatus makes it an even better fit; accordingly the identity of specimens here designated as *D. calyciformis* is determined by comparison with this exsiccatus. Jørstad's, Schellenberg's, and Maublanc's measurements indicate that the fungus believed to be *D. calyciformis* today is probably the same as Rehm's *D. calyciformis.*

Kryptogamae exsiccatae *954* (1904), "*Dasyscypha calyciformis* Rehm apud Rabenh." (NY.); containing ten to fifteen good apothecia which are both overmature and molded, as well as a few imperfect fruiting bodies. The overmaturity and weathering have contributed in making the ascospores irregular in shape; otherwise

the perfect stage of this exsiccatus fits closely the perfect stage of Rehm's *D. calyciformis* as exemplified by his *1163*.

Kryptogamae exsiccatae *1821* (19??), *"Dasyscypha calyciformis* Rehm apud Rabenh." (NY.); containing ten to fifteen good apothecia and several imperfect fruiting bodies. It compares closely with the perfect stage of the Rehm exsiccatus in all respects.

Krieger, Fungi saxonici *916* (1894), *"Dasyscypha calyciformis* (Willd.) Rehm" (NY.); containing a few good but severely weathered apothecia and no imperfect fruiting bodies. Despite the weathered condition the characters of the perfect stage are quite similar to those of the Rehm exsiccatus.

Jaczewski, Komarov, & Tranzschel, Fungi rossiae exsiccati *244* (1896), *"Dasyscypha calyciformis* (Willd.) Rehm" (NY.); containing a generous number of well preserved apothecia but no imperfect fruiting bodies. The perfect stage is quite similar to the perfect stage of Rehm's exsiccatus.

Exsiccati apparently incorrectly designated or cited as *D. calyciformis:* Allescher & Schnabl, Fungi bavarici *169* (1891), *Dasyscypha calycina* (Schum.) Fries" (Path. & Myc. Coll., U. S. D. A., Wash., D. C.: BPI.), cited by Rehm (**1893**: 834) as representing *D. calyciformis;* de Thümen, Mycotheca universalis *1508* (1880), *"Dasyscypha calycina* Fuck." (BPI.), cited by Rehm (*loc. cit.*) as representing *D. calyciformis;* D. Saccardo, Mycotheca italica *672* (1901), *"Dasyscypha calyciformis* Sacc." (NY.); Sydow, Mycotheca germanica *905* (1910), *"Dasyscypha calyciformis* (Willd.) Rehm" (Mo. Bot. Gard. Herb., St. Louis, Mo.: MO.).

Exsiccati of uncertain identity: Rabenhorst, Herbarium mycologicum *422* (1857), (BPI.), cited by Rehm (*loc. cit.*) as representing *D. calyciformis,* but "material of no value" teste Edith K. Cash; Rabenhorst-Winter, Supplement, Fungi europaei *1422b* (1871), "*Dasyscypha calycina* Fries" (BPI.), cited by Oudemans (**1919**: 367) as representing *D. calyciformis,* but Rehm (**1893**: 834) cites, with question, only the exsiccatus *1422* which is apparently distinct from the supplement *1422b*.

Other specimens apparently incorrectly designated as *D. calyciformis:* Michigan State College Herb. (MICH.) "Fp *78*"; C. R.

Stillinger *1412, 2297,* & *2602;* J. R. Hansbrough *578* & *579* (NY.); University of Idaho Forest Pathology Herb. *1341, 1347, 1467, 2202,* & *2829.* None of these eleven American collections, determined or tentatively determined as *D. calyciformis,* is considered to be correctly designated on the basis of comparison with the Rehm exsiccatus (Ascomyceten *1163*).

HOSTS AND GEOGRAPHICAL RANGE

The five correctly designated exsiccati which have been examined are all on *Abies* from Austria, Germany, Hungary, or Russia.

A fairly comprehensive literature survey reveals that *D. calyciformis* ranges widely over the European countries where it occurs most frequently on *Abies.* Jørstad (**1931**: 78–96) reported the fungus on *A. alba* Mill., *A. balsamea* (L.) Mill., *A. cephalonica* Loud., *A. concolor* Lindl. & Gord., *A. Fraseri* Poir., *A. grandis* Lindl., *A. nobilis* Lindl., *A. nordmanniana* Spach., and *A. sibirica* Ledeb. in Norway. Falck (**1927**: 397–409) and Plassmann (**1928**: 272–283) reported the fungus on *Abies* in the Eifel district of Germany, Plassmann (**1927**: 18–19) having previously reported the fungus on *Pinus Strobus* L., *P. sylvestris* L., and *Picea* at Hann.-Münden, Germany. Schellenberg (**1905**: 269–286) reported the fungus on *Abies sibirica* and *A. alba* from Adlisberg, Germany; while Rehm (**1893**: 834–835), in redescribing the species, reported it on branches of *A. alba, Pinus Mugo* Turra var. *pumilio* Zenari, and (?) *Pinus larix* from Switzerland, Germany, and Austria. Ferdinandsen (**1928**: 275) reported the fungus on *Pinus contorta* Dougl., *Picea abies* Karst., and *P. sitchensis* (Bong.) Carr. from Denmark. Zederbauer (**1906**: 1) reported the fungus on *Picea abies* Karst. from Bohemia and Austria. Maublanc (**1904**: 235) reported the fungus on *A. alba, Larix,* and *Pinus* from France; Vuillemin (according to Maublanc) reported it on *Picea,* also from France. Noack (**1928**: 707) reported it on *Pinus Mugo* and *Larix decidua* Mill. Wilson (**1921**) reported the fungus from Great Britain, Feltgen (**1903**: 66) from Luxemburg, Strasser (**1902**: 435) from northern Hungary, and Ludwig (**1894**: 337–338) from Graz, Austria. None of these last four publications has been seen even in satisfactory abstract form so the hosts have not been ascertained.

It would seem then that, although *Abies* is most frequently the host upon which *D. calyciformis* occurs, the fungus may also occur on *Pinus, Larix,* and *Picea* with some frequency. The fungus is presumably European in geographic range as all the records and specimens which have been seen come from the European countries. However, lists from continents other than North America and Europe have not been examined.

ILLUSTRATIONS SEEN

Rehm in Rab. Krypt.-Fl. **1** (3): 825, *fig. 7.* 1893; Maublanc in Bull. Soc. Myc. Fr. **20**: 235, *fig. 1–8.* 1904; Schellenberg in Schweiz. Centralanst. Forstl. Versuchw., Mitt. **8**: *pl. I, fig. 1–3; pl. II, fig. 1–13.* 1905; Plassmann, Untersuchungen über den Lärchenkrebs, *fig. 5–8.* 1927.

PARASITISM

European mycologists who have studied *D. calyciformis* seem to consider it, except in a few cases, saprophytic or mildly parasitic. Thus Jørstad (**1925**: 39 & 116) considered the fungus a saprophyte on *Pinus sylvestris* although of some importance, but not producing real cankers, on *Abies,* especially *A. balsamea.* Jørstad (*ibid.:* 116) implied that it is only weakly parasitic as he reported that it is often found associated with dead and dying *Abies* but is mainly saprophytic. Falck (**1927**: 397–409) reported that he found *D. calyciformis,* with other fungi and an aphid, involved in a die-back disease of *Abies;* but Plassmann (**1928**: 272–283) later reported that the disease was largely due to the insect attack and that the fungi, including *D. calyciformis,* were acting in a saprophytic capacity, evidently secondary to the attack of the aphid. Zederbauer (**1906**: 1) reported *D. calyciformis* as a wound parasite of considerable frequency on *Picea,* while Ferdinandsen (**1928**: 275) reported it as a wound parasite causing damage on artificially regenerated *Pinus contorta* but of small importance on *Picea abies* and *P. sitchensis.* Maublanc (**1904**: 233) reported that he had found the fungus on bark already parasitized by *Armillaria mellea* (Vahl ex Fries) Fries; Vuillemin (according to Maublanc) reported the fungus to be a saprophyte, developing in wounds. Schellenberg (**1905**: 284–286) found that the fungus

caused inconsequential damage on suppressed or injured twigs of *A. alba* but severe damage on vigorous branches and trunks of *A. sibirica.* Noack (**1928**: 707), following Wagner (**1896**: 321), said it was usually found as a saprophyte, but sometimes as a parasite, especially in young trees and on damp sites.

DISCUSSION

It is realized that the value of the foregoing description is limited by the small number of specimens available for study, none of them fresh. The description, in fact, is based on five exsiccati of which only one (Rehm, Ascomyceten *1163*) can be considered as determinative. Examination of these few exsiccati has not provided a complete understanding of Rehm's fungus but the description is believed adequate for distinguishing the other *Dasyscyphae* under consideration from this fungus. It is not known whether a type specimen exists today because collections in European herbaria have not been available for study. A recent letter from Miss E. M. Wakefield merely reports the absence of any Rehm specimens of this species in the Herbarium of the Royal Botanic Gardens at Kew (K.).

Information concerning hosts, geographical range, and parasitism is taken from the few specimens examined and from the fairly completely covered literature. The difficulty in obtaining herbarium specimens from countries abroad and the limited time available for literature survey prohibit a more exhaustive study of these features at this time.

D. calyciformis, as exemplified by the Rehm exsiccatus, is also believed distinct from other small- and large-spored *Dasyscyphae.* Authentic materials of the small-spored species, *D. resinaria* (Cooke & Phill.) Sacc. and *D. subtilissima* (Cooke) Sacc., have not been examined. The published descriptions, however, show these species to differ from *D. calyciformis* in ascospore and ascus size as well as in other morphological features. Jørstad (**1925**: 39) has only recently verified the differences in ascospore size. Hahn and Ayers (**1934**) have thoroughly described the large-spored species *D. calycina* Fuckel and *D. Willkommii* (Hartig) Rehm, and Plassmann (**1927**: 18) and Schellenberg (**1905**: 270)

have shown in convenient tabular summaries how the asci, ascospores, and paraphyses of *D. calyciformis* differ from those of *D. Willkommii,* so that there should be no confusion with these species. Noack (**1928**: 707) pointed out that the discs of *D. calyciformis* are not orange as in other species, but pure yellow.

The imperfect stage, as far as can be determined, has never been assigned to a form-genus nor given a specific name. Maublanc (**1904**: 234) and Schellenberg (**1905**: 274) pointed out that Rehm (**1893**: 835) was in error when he suggested *Phoma abietina* Hartig as the imperfect stage.

DASYSCYPHA AGASSIZII

Among the small-spored American *Dasyscyphae, D. Agassizii* (Berk. & Curt.) Sacc. is perhaps the most widely found and universally recognized. According to Snell (**1929**: 242) it is fairly closely limited to the northeastern part of this continent. As determined from many specimens in NY., the Farlow Herb. (FH.), the Brown Univ. Herb. (BRU.), the Dodge Herb. (then at Harvard), the Overholts Herb. (Penn. State College), and BPI., he reported the geographical range to be sub-boreal (north of 43° 30′ north latitude) with only a few specimens from west of the Mississippi River. Snell (*ibid.:* 235–242) also reported that *D. Agassizii* was frequently found on blister rust cankers of *Pinus Strobus* L. in New York. In so doing, Snell raised a question in the minds of northwestern collectors as to whether the similar small-spored *Dasyscypha* on blister rust cankers of *P. monticola* (described later in this paper) might not also be *D. Agassizii.*

The literature shows that the morphological characters of *D. Agassizii* vary among individual specimens. Thus Berkeley (**1875**: 151), in his original publication of the species, described the ascospores as narrowly oblong or subfusiform, about 5.1 μ (.0002″) long. In the Sylloge, Saccardo (**1889**: 438) described the ascospores as ellipsoid-ovate or subfusiform, 6.5–7.5 $\times$ 4 μ in size. Snell (*op. cit.:* 240) reported the ascospores as large as 10.5 $\times$ 5.25 μ (on *Abies*) and as small as 6.5–7.5 $\times$ 3–3.5 μ (on *Picea*).

Dasyscypha Agassizii, as far as can be determined, has not been redescribed since Saccardo (**1889**: 438) included it in the Sylloge.

Because there is a difference of opinion in the literature as to spore size and shape, and because it has been confused with the two other small-spored *Dasyscyphae* discussed in this paper, authentic material has been studied. An amended description, based on thirty specimens including a slide of the isotype specimen, follows.

DASYSCYPHA AGASSIZII (Berk. & Curt.) Sacc. Syll. Fung. **8**: 438. 1889.

Peziza (*Humaria*) *Agassizii* Berk. & Curt. in Grevillea **3**: 151. 1875.

Apothecia abundant, subphellar or shallowly intra-phellodermal, erumpent, gregarious or solitary, often several from a common subicle-like base; *stipes* moderately long to long, up to 2.5 mm., each with from one to as many as a dozen cups; *cups* when young tightly closed, with age opening circularly and expanding under moist conditions to a disc-like form with a very thin, undulating margin, drying circularly when young but with age drying irregularly either by a partial inward curling or by folding across the disc, externally whitish with excipular hairs; *hairs* cylindrical, thin-walled, moderately to considerably roughened by minute tubercles, with unswollen or slightly swollen, rounded, occasionally sub-acute tips, noticeably septate at 10–25 μ intervals, 2.5–4.5 μ wide, always present on the younger apothecia but not persistent, often entirely missing except at the margins on the older apothecia (FIG. 2, *C*); *discs* yellow to bright orange, large, 0.5–5.5 mm. wide, mostly 1.5–4.0 mm. wide; *hyphae* of the context variable in width, 2.0–6.0 μ wide, septate, irregularly swollen, much branched at various angles. **Asci** cylindrical to clavate, with rounded or sub-acute apices, size range (250) 43.5–67.5 $\times$ 4.0–6.0 μ, commonly 47–60 $\times$ 4.5–5.5 μ (FIG. 2, *A*). **Ascospores** eight, *arrangement* uniseriate, oblique with the basal spores almost vertical, occasionally irregularly arranged or with a change in the direction of the oblique arrangement, rarely biseriate; enclosing *membrane* usually flattened or slightly concave, not following the contour of the ascus at the apex; *shape* usually elliptical to plump-ovate, extreme range from subfusiform to almost spherical, occasionally with one end (the lower when in the ascus) slightly more acute or collapsed; *walls* thin; *contents* hyaline, indistinctly nucleate and either biguttulate or uniguttulate, continuous (germination not observed) or rarely uniseptate both inside and outside the ascus; *size* range (660) 5.0–9.5 $\times$ 2.0–4.0 μ, commonly 5.5–8.5 $\times$ 2.5–3.5 μ (FIG. 2,

A & *B*). **Paraphyses** usually exceeding the asci by from 5 to 15 μ, relatively stout and coarse, smooth, occasionally branching near the base; *shape* usually distinctly swollen to almost spatulate at the tips, tapering more or less sharply past other swellings in the upper portion, to the base, the swollen paraphyses intermixed with smaller

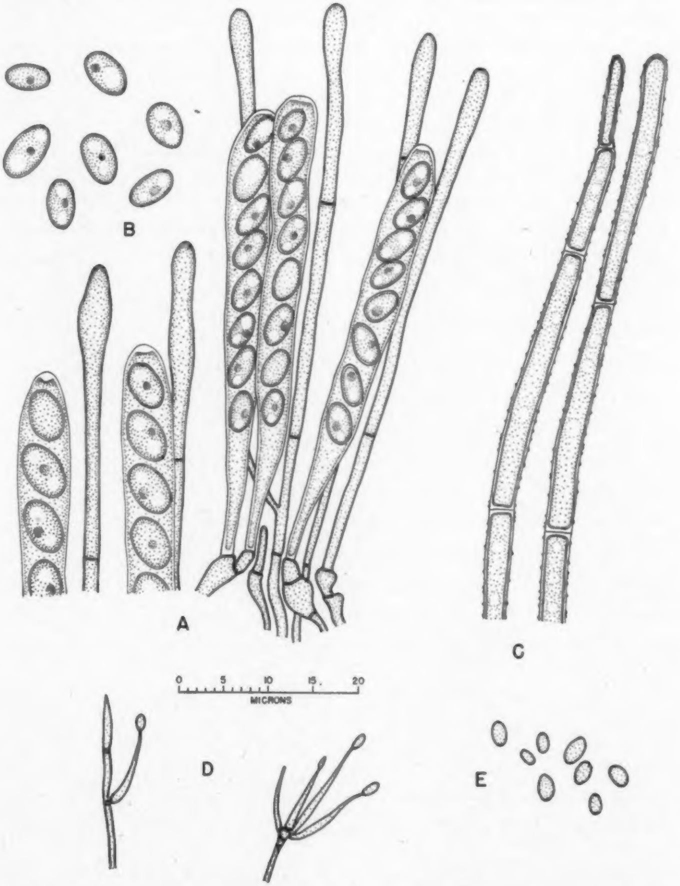

FIG. 2. *Dasyscypha Agassizii* (Berk. & Curt.) Sacc. Drawings made with aid of a micro-projector; those of the perfect stage from Walter H. Snell *703* and *1825*, those of the imperfect stage from L. O. Overholts *4841*. *A*, asci and ascospores; *B*, ascospores; *C*, excipular hairs; *D*, conidiophores and phialides; *E*, conidia.

numbers of shorter and more slender, cylindrical paraphyses, tips rounded or sub-acute; *contents* obscurely or clearly septate, minutely guttulate; *size* range (240) 47.5–94.5 × 1.0–3.5 μ (at the widest point), commonly 65–85 × 2.0–3.0 μ (FIG. 2, *A*).

Imperfect fruiting bodies very infrequently found, inconspicuous, developing from light-colored, sub-phellar, erumpent stromata; with age becoming multi-loculate, and the outer wall and overlying bark more or less completely lost. **Conidiophores** erect, entirely lining the locules, simple or freely branched, noticeably septate, hyaline, minutely guttulate; bearing sparsely at the sides, and sparsely or profusely at the apices, the unicellular, curved, moniliform or only slightly subulate spore-bearing elements (phialides). **Conidia** abstricted from the acute apices of the phialides, hyaline, continuous (germination not observed), very thin-walled, once seen exuded from the top of a mature stroma in a whitish, translucent mass, usually elliptical or ovate, size range 1.5–4.5 × 0.5–3.0 μ (FIG. 2, *D* & *E*).

TYPE SPECIMEN

Berkeley (**1875**: 151) designated no type specimen but cited two Curtis Herbarium numbers: "On bark. Lake Superior, Agassiz. No. *2631.* New England, Oakes. No. *3098.*" Snell (**1929**: 238) has pointed out that the host of *2631* was indicated by Curtis as "in cort. Pini" and by Saccardo as "in corticibus Abietis"; a slide prepared from this number (FH.) by Hahn and Ayers is labelled "on Pine." The New England specimen (*3098,* FH.) is labelled "trunc. Abietis?" in Curtis' handwriting and Snell (*loc. cit.*) has confirmed this as *Abies balsamea.* Miss Wakefield (**1942**: Personal communication) reported merely that "there is a type specimen" at Kew.

In view of these circumstances, Curtis' *2631* at Kew may be designated as the type specimen, *3098* at Kew as the paratype,[8] *2631* at the Farlow as the isotype,[8] and *3098* at the Farlow as part of the paratype. Alternatively, both *2631* and *3098* at Kew may be regarded as type specimens and those at the Farlow as isotypes.

SPECIMENS EXAMINED

Isotype specimen: Curtis Herb. *2631, "Peziza Agassizii* B. & C." (FH.); now containing only two apothecia—teste Dr. Linder.

[8] After Ewan (**1942**: 8–9).

The specimen itself was not seen, but a slide of the perfect stage containing ascospores, asci, paraphyses, excipular hairs, and context hyphae, made by G. G. Hahn and T. T. Ayers in 1931, was kindly loaned by Dr. Linder. This slide served to verify the opinion that *D. Agassizii* is a distinct species as it contains all the features of the perfect stage, notably the flat-topped ascus membrane and the swollen paraphyses tips, believed diagnostic for this species. Hahn's (**1942**: Personal communication) measurements of ascospores, presumably from this slide, were given as 5.8–8.9 × 3.0–4.4 μ. Measurements of ascospores from the same slide, made during this study, differed, being (40) 4.5–9.0 × 2.0–4.0 μ. The smaller length and width of the latter measurements is probably due to the manner in which ascospore measurements were taken throughout the study; *i.e.*, no satisfactory means for determining the maturity of ascospores outside the ascus having been found, the ascospores were measured as encountered in the microscope field, avoiding only collapsed or distorted spores.

Exsiccati apparently correctly designated as *D. Agassizii:* Rehm, Ascomyceten *1854* (1909), *"Dasyscypha Agassizii* (B. & C.) Sacc." (NY.), on *Abies balsamea* from Wisconsin; Ellis, North American Fungi *1311* (1885), *"Peziza Agassizii* B. & C." (three specimens: from the Ellis Collection, from the Columbia College Herb., and from the Underwood Collection; all in NY.), on dead limbs from New Hampshire.

Other specimens apparently correctly designated as *D. Agassizii:* W. S. Snell *703, 1459, 1586,* & *1825;* W. G. Farlow (NY., & MO. *18433*); L. M. Underwood, North American fungi (two specimens from the Underwood Collection, NY.); W. A., & E. L. Murrill, Fungi of Lake Placid *261* (NY.); L. O. Overholts *4841* (MO. *55828*); B. L. Robinson & H. Schrenk, Flora of Newfoundland, distributed by the Harvard University Herb. (NY., & MO. *18268*); J. B. Ellis & A. C. Waghorne, Newfoundland fungi *65* (MO. *18428*); H. C. Peck & A. C. Waghorne, Labrador fungi *18* (MO. *18429*); University of Toronto Cryptogamic Herb. *7908* (NY., & Oregon State College Herb. (OSC.) *9802*); MO. *150902;* J. Ehrlich *841, 843, 844, 922, 981,* & *1116.*

Other specimens apparently incorrectly designated as *D. Agassizii:* C. R. Stillinger *2221;* E. E. Honey (NY.); MO. *18430* &

55451; Wm. Bridge Cooke *67* (MO. *156204*); University of Idaho Forest Pathology Herb. *2058*.

Other specimens of uncertain identity: S. M. Zeller *6799* (*"Dasyscypha Agassizii* (Berk. & Curt.) Sacc. var. *rufipes* Phill."); W. A., & E. L. Murrill, Fungi of Lake Placid *554* (NY.) (lacks apothecia).

HOSTS AND GEOGRAPHICAL RANGE

Specimens have been examined on *A. balsamea, A. Fraseri,* and *Abies* sp. from Maine, New Hampshire, New York, North Carolina, Wisconsin, Labrador, New Brunswick, Newfoundland, Nova Scotia, and Ontario; on *Pinus Strobus* from New York; and on unknown hosts from New Hampshire and New York.

Snell (**1929**: 236–240) collected *D. Agassizii* on *Picea rubra* in New York; and reported specimens in NY. on *Abies balsamea* from Pennsylvania, and in the C. W. Dodge Herb. on *A. balsamea* from Quebec. Snell also reported specimens in FH. on *Tsuga canadensis* (L.) Carr., in NY. on *Picea, Pinus,* and *P. monticola,* and in BPI. on *Picea mariana* (Mill.) Britt. et al. without giving the geographical range; specimens in FH. from Vermont, and in BPI. from Massachusetts, Montana, and Idaho without giving the hosts. Seymour (**1929**: 30 & 49) reported *D. Agassizii* on *Pinus contorta* and on *Larix laricina* (Du Roi) K. Koch. Bisby (**1938**: 39) reported *D. Agassizii* on *Abies balsamea* from Manitoba.

It is believed that most of the northwestern material designated as *D. Agassizii* is incorrectly determined, as none of the northwestern material examined during this study has been found to be characteristic. *D. Agassizii,* then, seems to be found most frequently on *Abies,* particularly *A. balsamea;* next most frequently on *Pinus,* particularly *P. Strobus;* and infrequently (not verified) on *Larix, Picea,* and *Tsuga.* In geographical range it appears to be limited to the northeastern part of this continent, occasionally (not verified) in outlying western states.

ILLUSTRATIONS SEEN

Cooke in Grevillea **3**: *pl. 40* (*fig. 169*). 1875.

PARASITISM

Snell's (**1929**) report of *D. Agassizii* on presumably live blister rust cankers of *Pinus Strobus* and Ehrlich's (**1932**: 38) report of *D. Agassizii* on weakened *Abies balsamea* are the only discovered references to this fungus occurring on live tissues. All specimens examined either stated on their labels that the host was dead or failed to record the host relationship. *D. Agassizii* would seem to be only a weak parasite acting as a secondary fungus on already weakened tissues or, most often, a pure saprophyte.

DISCUSSION

The amended description given, based on thirty specimens including an isotype specimen, serves to distinguish *D. Agassizii* from its close relatives, the European *D. calyciformis* and a *Dasyscypha* found on *Pinus monticola* in the Northwest. The principal distinguishing characters of *D. Agassizii* were found to be the size of the ascospores (slightly larger than in either of the other species under consideration), the often swollen tips of the paraphyses (not swollen in either of the other species), and the flat-topped ascus membrane (following the contour of the ascus in the other species). It may be distinguished from other small-spored *Dasyscyphae* on the basis of ascospore size and shape alone. It possesses an infrequently found imperfect stage like that in *D. calyciformis*.

(To be concluded in a later issue)

TAXONOMY, DISTRIBUTION, AND PATHOLOGY OF PHOMOPSIS OCCULTA AND P. JUNIPEROVORA

Glenn Gardner Hahn

(with 2 figures)

INTRODUCTION

The heightened esteem of soil conservationists and the public generally, particularly in the Central States, for the eastern red cedar (*Juniperus virginiana* L.) is causing forest pathologists to focus their attention on diseases affecting the common cedar, the importance of which has increased greatly because of its present extensive use in planting projects. Red cedar is considered by many as being one of our finest ornamentals. In the Prairie States its value for farmstead windbreaks is recognized now as never before.

For approximately 50 years pathologists have been interested in a destructive nursery disease of the native red cedar known as cedar blight, which is caused by *Phomopsis juniperovora* Hahn. During the early period of the investigation of this juvenile disease from 1895 to 1920 (**3**, **9**, **10**, **15**) a closely related *Phomopsis* was unknown. A few years later, however, such an organism entered the pathological picture and became a complicating factor in the identification of the nursery parasite. When first recognized the related *Phomopsis,* which was demonstrated by the writer to be nonpathogenic on two members of the Cupressaceae, was regarded as a physiological variety of the cedar-blight organism (**4**). Later investigations showed it could be distinguished morphologically from *P. juniperovora,* and he identified it as *P. occulta* (Trav.) Sacc., while studying the genus *Phomopsis* on conifers in Great Britain during 1926 to 1929 (**3**). Since *P. occulta* is widely distributed in the United States and since it is to be found on dead branchlet tips of wildling as well as nursery stock of east-

ern red cedar, its presence has confused the identification of the true cedar-blight pathogen on both kinds of stock unless careful laboratory studies are made.

The control of cedar blight continues to be a most refractory problem in Federal and commercial nurseries of the States of the Mississippi Valley and in the Southeast. Inasmuch as our present knowledge of the life history of the causal organism on wildling junipers and its dissemination from this source is limited, it was important that information be obtained on the related *Phomopsis occulta* in order to determine whether or not it was to be considered a potential factor in nursery infection. Accordingly an investigation [1] of *P. occulta* on eastern red cedar was carried out, the results of which demonstrated its nonpathogenicity on this valuable conifer.

INOCULATIONS WITH PHOMOPSIS OCCULTA AND P. JUNIPEROVORA

The investigation of *Phomopsis occulta* was concerned with the problem of determining whether it was parasitic to any degree upon wildling red cedar. Comparable tests were performed at the same time with the cedar-blight parasite, *P. juniperovora,* which were used as a "check" to establish the suitability of the environmental conditions for the experiments. The inoculations were performed June 16 to 18, 1941 on vigorous 3- to 6-year-old potted stock of the wildling reproduction of eastern red cedar that had been obtained earlier in the spring in the field near Durham, Connecticut. The tests were carried on in an unheated greenhouse in the Marsh Botanical Garden, Yale University, New Haven. The cedars, which for the most part averaged 30 inches in height, apparently were unaffected by transplating. Some of them showed the presence

[1] Acknowledgment is made by the writer of assistance rendered during this study by the following investigators: to Miss Edith K. Cash, for examining North American *Diaporthae* on conifers deposited in the collections of the Division of Mycology and Disease Survey; to Dr. G. H. Hepting, Division of Forest Pathology, Bureau Plant Industry, for permission to report unpublished data dealing with *Phomopsis occulta;* and to Dr. A. J. Riker, University of Wisconsin, Dr. F. L. Howard, Rhode Island State College, and Messrs. W. C. Davis, D. H. Latham, and G. Y. Young, and Dr. E. Wright of the Division of Forest Pathology, for specimens of diseased wildling and nursery red cedar stock.

of numerous galls of the cedar-apple rust, *Gymnosporangium juniperi-virginianae* Schw.

The inocula utilized for the tests consisted of monospore cultures of *Phomopsis juniperovora* and *P. occulta* growing on synthetic malt agar. The former was isolated from pycnidia that occurred abundantly on badly diseased Rocky Mountain juniper (*Juniperus scopulorum* Sarg.) nursery seedling stock collected at a Federal nursery at Fremont, Nebraska, September 1940. The latter was obtained also from pycnidia on eastern red cedar from two sources, namely, a dead branchlet tip of diseased 3- to 5-year-old nursery stock collected in Maryland near the District of Columbia, January 6, 1941, and a similar branchlet taken from wildling material collected at Quissett (Falmouth), Massachusetts, February 1, 1941. In these two instances *P. occulta* appeared sparingly and occurred along with other fungi, *e.g.*, in the Maryland material a species of *Fusarium* occurred abundantly, whereas in the Massachusetts material *Cytospora* and *Pestalozzia* were observed among other saprophytes. In both cases the two collections had been submitted to the writer for diagnosis with the possibility that the *Phomopsis* might be the cedar-blight pathogen. Two individual but culturally similar isolates of each of the three collections mentioned above were used in the tests.

The technique employed in making the wound inoculations, which were placed only on the trunk, has been described in a previous paper (**4**). A single check incision was placed on each tree below the inoculated incisions.

RESULTS WITH P. OCCULTA

A total of 14 saplings were tested with *Phomopsis occulta* with the following pure cultures: Maryland collection—6 trees with the first isolate and 2 trees with a second isolate; Massachusetts collection—4 trees with the first isolate and 2 trees with a second isolate. All of the plants failed to show cankering or a blighted condition and both inoculated and check incisions healed without

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FIG. 1. Negative results were obtained in two inoculations with *Phomopsis occulta* on this eastern red cedar wildling which was infected naturally with cedar-apple rust. The inoculated incisions and the check below are indicated by arrows. (Photographed by H. G. Eno, March 1942.)

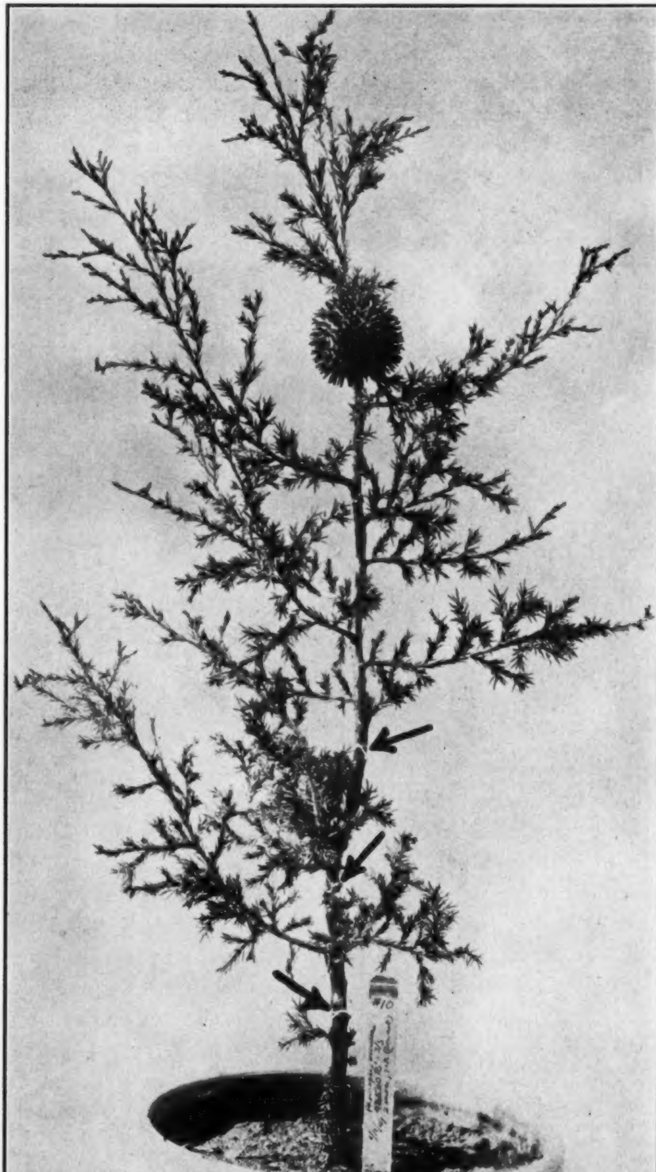

FIG. 1.

any fungus infection whatsoever. Included among the plants was a weakened sapling showing a large cedar-apple gall on its main stem. Despite the presence of this abnormality, the diseased condition of the wildling did not predispose it to infection of *P. occulta* (FIG. 1).

During the tests there appeared a natural colony of *P. occulta,* which was discovered in February 1942 growing below a colony of *Pestalozzia* at the tip of a very small dead branchlet apparently dead from natural causes. It is not known whether *P. occulta* was brought into the greenhouse from the field on the plant itself or whether it came from inoculum already present in the vicinity of the greenhouse where in the past the fungus has been collected on dead twigs of Douglas-fir (*Pseudotsuga taxifolia* (Poir.) Britton). Monospore cultures of the cedar collection of *P. occulta* showed agreement with isolates of the saprophytes used in the foregoing tests.

RESULTS WITH P. JUNIPEROVORA

A total of 7 saplings was tested with the cedar-blight parasite, 5 trees being inoculated with the first isolate and 2 trees with a second isolate. All of the saplings tested were 5 to 6 years old and one of them, a forked plant, was tested similarly with both species of *Phomopsis,* the larger shoot carrying two *P. juniperovora* inoculations. A total of 14 inoculations were made and all of them showed cedar-blight infection but in varying degrees.

Five saplings for which the first isolate was used were girdled at all except one of the incisions. In three of the trees, which were of a distinct bluish-green color and which appeared to be most susceptible, the parasite by March 20, 1942 had progressed downwards in the cortical tissues from the lower incision, 4 cm., 5 cm., and 6 cm., respectively. The forked tree of this group was inoculated with both *Phomopsis* species; *Phomopsis occulta* failed to infect the slenderer shoot (FIG. 2), whereas the stouter shoot was attacked by the pathogen. The terminal of this shoot was girdled and killed by the upper infection but a nongirdling canker at the lower incision affected only the lateral branches on one side of the trunk above the inoculation (FIG. 2).

The two remaining cedars, which were of a lighter green color, were tested with a second isolate, and evinced cedar-blight resistance. One of these showed a blighted tip that had resulted from a girdling infection at the upper incision, whereas at the lower incision, a limited nongirdling canker formed and the laterals involved by it were not discolored. The other tree, which was lighter and brighter in color than the other, showed nongirdling cankers but no discoloration of lateral branches. It is here of interest to note that the "Hill Dundee" juniper (*Juniperus virginiana* var. *pyramidiformis* D. Hill), reported as being highly resistant to cedar blight (**6**, p. 56), is described by Rehder (**13**) as having also bright green foliage in summer.

One of the tested cedars, which was bluer in color than any of the others, was the first to show external evidence of disease. By mid-July its terminal was brown and lateral branches below were becoming discolored. By September 24, 1941 the parasite had extended its growth downward 6.5 cm. from the lower inoculation to invade the check incision. *Phomopsis juniperovora* was reisolated from this wildling on February 21, 1942 at which time the pathogen was recovered in a pure condition 8 cm. below the lower inoculation, and also at a point midway between the two inoculations placed on the main trunk, 10.5 cm. from each incision. The reisolated parasite produced the yellow coloration accompanied later by flaming orange crystals (**3**, **5**, **9**) in a synthetic malt substratum, both color and crystals being characteristic of the physiological activity of *P. juniperovora* growing on a number of agar media. A fine blackened line or zone within which perithecia of *Diaporthe* may be formed was observed in the cortex 2 cm. above the lower incision, similar to that described and illustrated by Wehmeyer (**18**, pl. III).

It was not possible to reisolate *Phomopsis juniperovora* from the dead tissues of the slender terminal distal to the upper inoculation on this very susceptible blue wildling. Moreover, no fruiting bodies of the parasite were found on the trunk or the dead laterals killed by girdling. The laterals were colonized, however, by secondary fungi—*Botrytis, Alternaria, Pestalozzia,* etc. Pycnidia, moreover, were not observed on any of the other trees tested with the cedar-blight parasite. In earlier studies the writer obtained

fruiting bodies of *P. juniperovora* on inoculated 2-year-old red cedar nursery stock (**9**, pl. 61).

DISCUSSION OF THE TAXONOMY, DISTRIBUTION, AND PATHOGENICITY OF THE TWO RELATED PHOMOPSIS

Because of the close morphological similarity between *Phomopsis occulta* and *P. juniperovora,* the distribution of the latter as an endemic parasite on the common red cedar has been confused. This has been especially true where diagnoses of the pathogen have been made on wildling junipers.

P. occulta

A previous biometrical study made by the writer (**5**, p. 56) of the size of A-spores of *Phomopsis occulta* distinguished them from those of the cedar-blight pathogen. While there is overlapping of the spore size ranges of the two species, A-spores of *P. occulta* were found to be slightly longer than those of *P. juniperovora* and significantly different with regard to this character; the A-spores of the former gave a mean of 7.5 μ (collections from *Pseudotsuga*) and 7.2 μ (collection from *Thuja*), while those of the latter gave a mean of 8.7 μ (collections from *Juniperus*). At the same time the writer reported that the filamentous B-spores of *P. occulta* are inclined commonly to be bent at one end like a walking stick, whereas those of *P. juniperovora* tend to be straighter. Intermediate spores, moreover, are found more frequently in *P. occulta* than in *P. juniperovora.*

Where there is any doubt in the mind of the investigator concerning a morphological distinction, the two species of *Phomopsis* may be differentiated with certainty by cultural characters that are striking and constant. *Phomopsis juniperovora* produces yellow coloration in the medium accompanied by flaming orange crystals on synthetic malt agar as well as on a number of other media (**3**, **5**, **9**); neither the yellow color nor the crystals appear in cultures of *P. occulta* on the same media.

While in Great Britain in 1926 to 1929 (**5**, pp. 75–76), the writer investigated the life-history of *P. occulta* and demonstrated that it belonged to *Diaporthe conorum* (Desm.) Niessl (syn. *D.*

occulta (Fuckel) Nit., *D. pithya* Sacc.). According to Wehmeyer (**18**) *Diaporthae* on conifers including *D. conorum, D. occulta, D. pitya, D. pinophylla* Plowr. & Ph., *D. pinicola* Hazl., *D. conigena* Feltg., and probably *D. thujana* Petr. are all forms of *D. eres* on conifers. The last named *Diaporthe* described on *Acer* is given by Nitschke as the type of the genus, and because of this Wehmeyer (**18**) has retained its name for an exceedingly large species complex of related host forms although *D. conorum* holds priority. The fact that the writer succeeded in obtaining fruiting bodies of *D. conorum* on sterilized twigs of English elm (*Ulmus procera* Salisb.),[2] from monopycnidiospore cultures derived from monoascospores taken originally from Douglas-fir, upholds in a measure Wehmeyer's opinion of the relationship of the conifer species to *D. eres*.

In the United States the writer has collected or identified *Phomopsis occulta* on a wide range of conifer hosts in both the eastern and western sections of the country (**4**). These hosts are represented by the following genera: *Abies, Cephalotaxus, Cryptomeria, Cupressus, Juniperus, Larix, Picea, Pinus, Pseudotsuga, Sequoia, Taxodium, Taxus, Thuja,* and *Tsuga*. In western Europe *P. occulta* is also widely distributed on numerous conifer hosts (**5**).

The perfect stage, *Diaporthe conorum,* also may be collected commonly in Europe whereas in North America it occurs apparently with great rarity, if at all. Although the writer was able to find the perfect stage without difficulty in Great Britain, particularly in plantations of the exotic Douglas-fir, he has not come upon *Diaporthae* on conifers including Douglas-fir in this country despite extensive observations.

Wehmeyer's (**18**) studies of *D. eres* on conifers dealt with European specimens, some of which were sent him by the writer from Great Britain. There is one exception, for Wehmeyer (**18**, p. 237) did report the species *D. disputata* Bomm., Rouss. & Sacc. (Ellis Herb. Myc. 189. 14 (2048), juniper, London, Ontario, Nov., 1903), which he stated is probably a form of *D. eres* (ascospores, 9.5–15 × 2.5–4 μ) on conifers. This juniper species and *D. thu-*

[2] Data contained in an unpublished manuscript entitled "Life-history studies of the species of *Phomopsis* occurring on conifers. Part II. *Diaporthe conorum* (Desm.) Niessl" by G. G. Hahn.

jana on *Thuja,* both described originally from European material, show ascospores that are slightly larger (12–17 × 3–5 μ) than the other *Diaporthae* on conifers.

Seymour (**14**) cited only two *Diaporthae* on conifers in North America, *Diaporthe griseotingens* (Berk. & Curt.) Sacc. and *D. disputata* on *Juniperus virginiana.* Wehmeyer (**18**, p. 254) reported the type of the former to be a *Physalospora* (*P. Cupressi?*). Moreover, in the collections of fungi deposited with the Division of Mycology and Disease Survey, Bureau of Plant Industry, all the specimens on conifers filed under *D. eres* or under the various other specific names listed by Wehmeyer (**18**) are European.

Inasmuch as *Diaporthe conorum* was collected commonly by the writer on Douglas-fir in Great Britain under environmental conditions very similar to those encountered in the Pacific Northwest, it was reasonable to expect that it would be found on native stands of that host in this country; for the imperfect stage of the species is present in the West on Douglas-fir, and inasmuch as the species is homothallic (**5**, p. 75), the presence of plus and minus strains is not required for the formation of the *Diaporthe* stage. However, to the best of the writer's knowledge *D. conorum* has not been collected in the Northwest. J. R. Hansbrough of the Division of Forest Pathology, who has collected widely in that locality, did not come upon the species.

In Europe *Diaporthe conorum* occurs as a saprophyte. This is also true of its imperfect stage, *Phomopsis occulta,* which the writer (**5**) found to occur as a secondary organism, particularly in cases of frost injury on such exotics as Douglas-fir. In this country his experience with *P. occulta* has indicated that generally speaking it is secondary following injuries caused by frost, transplanting, or drought, or those due to parasitic fungi, e.g., white pine blister rust (*Cronartium ribicola* Fisch.).

Contributory evidence of the nonpathogenicity of *Phomopsis occulta* on eastern hemlock (*Tsuga canadensis* (L.) Carr.) is given by the following investigators. G. H. Hepting of the Division of Forest Pathology at Asheville, North Carolina performed wound inoculation experiments in 1935 with the organism, isolated from diseased hemlock, on healthy plants of that species. Four tests made in October yielded negative results. Subsequently Hepting

proved that the hemlock twig blight was caused by the rust *Melampsora Farlowii* (Arth.) Davis (**11**). *P. occulta* was also identified by the writer on diseased hemlock material submitted by B. O. Dodge, New York Botanical Garden. Dodge (**2**) reported that he was unable to induce blighting by spraying dormant hemlocks brought into a greenhouse with spore suspensions of the *Phomopsis* and he concluded that the organism was secondary.

The negative tests of *Phomopsis occulta* on eastern red cedar reported in this paper corroborate similar results obtained in the earlier studies (**4**) with the same fungus on Arizona cypress (*Cupressus arizonica* Greene) and Chinese arborvitae (*Thuja orientalis* L.). Under nursery conditions both species may become quite susceptible to *P. juniperovora*. On the other hand the writer did obtain a certain amount of evidence that certain strains of *P. occulta* might become very weakly parasitic on Douglas-fir. In an early paper (**4**) he reported that under artificial conditions isolates of *P. occulta* obtained from diseased nursery stock of Douglas-fir and old ornamental trees of Irish yew (*Taxus baccata* L. var. *erecta* Loud.) produced a small amount of infection on potted saplings of the coast form of Douglas-fir.

P. juniperovora

The writer's experience with *Phomopsis juniperovora* has been confined almost entirely to investigations of the parasite on juvenile stock of members of the cypress tribe growing in Federal and commercial nurseries, and on nursery-grown shrubs transplanted to ornamental plantings. Eastern red cedar is the host most commonly attacked, upon which the disease in certain epidemic years may become exceedingly virulent. A large number of species of the Cupressaceae are known to be affected by the pathogen, a revised list of which is given:[3]

[3] Most of the host species recorded were determined by the writer although duplicate reports of some of them by other observers are reported. The susceptibility of *Juniperus pachyploea* is known only from an artificial test conducted by the writer (**9**). Infected species indicated by an asterisk have not been studied by the writer but were reported by other investigators. White (**19**) reported spiny Greek juniper (*J. excelsa* var. *stricta*) and Keteleer red redar (*J. virginiana* var. *keteleeri*) as being highly resistant to cedar blight. This is also true of the "Hill Dundee" juniper (*J. virginiana* var. *pyramidiformis*) (**6**, p. 56), which is not listed. The "Dundee" juniper has not been studied by the writer.

CHAMAECYPARIS—*Lawsoniana* (Murr.) Parl., *obtusa* (Sieb. & Zucc.) Endl., *pisifera* (Sieb. & Zucc.) Endl., *p.* var. *plumosa* (Carr.) Otto, *p.* var. *squarrosa* * (Endl.) Beiss. & Hochst.; CUPRESSUS—*arizonica* Greene, *a.* var. *bonita* Lemm. (*C. glabra* Sudw.), *Goveniana* Gord., *lusitanica* Mill. var. *Benthamii* (Endl.) Carr., *macrocarpa* Gord., *sempervirens* L., *s.* var. *stricta* Ait.; JUNIPERUS—*chinensis* L. var. *japonica* * (Carr.) Law., *c.* var. *mas* Gord., *c.* var. *Pfitzeriana* Spaeth., *communis* L., *c.* var. *sexatilis* Pall. (*c.* var. *montana* Ait., *J. sibirica* Burgsd.), *excelsa* Bieb. var. *stricta* * Gord., *horizontalis* Moench., *h.* var. *Douglasii* * Rehd., *lucayana* Britt., *mexicana* * (*J. ashei* * Buchh.) Schlecht., *pachyphlaea* Torr., *procumbens* (Endl.) Sieb. & Zucc., *Sabina* L., *s.* var. *tamariscifolia* Ait., *scopulorum* Sarg., *s.* var. *argentea* D. Hill, *squamata* Lamb. var. *Meyeri* * Rehd., *virginiana* L., *v.* var. *canaertii* Senecl., *v.* var. *Keteleeri* * Hort., *v.* var. *plumosa* * Rehd., *v.* var. *tripartita* Senecl.; THUJA—*occidentalis* L., *plicata* Lamb., *orientalis* L.

Under artificial conditions the writer in early studies (**4**, table 1) succeeded in obtaining positive infections of *Phomopsis juniperovora* on potted saplings of the Coast form of Douglas-fir upon which it was quite pathogenic. The Cupressaceous parasite therefore must be considered a potential enemy of this form of Douglas-fir, although to date the fungus is not known to occur on it naturally. Reisolates of the cedar-blight organism from infected Douglas-fir produced cultural characters identical with those exhibited by isolates of the fungus used as inocula, which were obtained from diseased stock of eastern red cedar and from that of Italian, Arizona, and Hinoki cypresses.

Cedar blight was first known as a destructive nursery parasite of eastern red cedar in Iowa (**15**). During the first two decades of the 20th century when the propagation and cultivation of ornamental conifers was on the increase in this country, the disease also was reported extremely serious on red cedar in commercial nurseries of States in the same region (**6**, Map). Since 1920 reports of the disease in nurseries of States of the eastern section of United States have accumulated but records of it in certain of them are meagre or lacking, and some of them are very recent. So far as is known to the writer, cedar blight has not been re-

ported in northern New England (Vermont, New Hampshire, Maine) or in the Southeast in South Carolina, Georgia, Florida and Mississippi. Very little is known about the disease in Louisiana. In the western section of the United States the nursery disease is known only in Nebraska, Kansas, and Oklahoma (recent reports); its occurrence in the Dakotas and Texas is problematical (**6**, **8**).

The writer has not studied material of *Phomopsis juniperovora* from the continent of Europe where it has long been suspected and has now been reported to occur as a pathogen on *Juniperus* and *Thuja* nursery stock (**16**, **17**). As in the case of *P. occulta,* its perfect (*Diaporthe*) stage, unknown in this country, may be present in Europe where it may occur on some obscure European host. There is also the consideration that *P. juniperovora* may be mutant, propagating itself only in the imperfect stage.

In this country not a great deal of information is available concerning the occurrence of *Phomopsis juniperovora* as an endemic parasite on mature trees and reproduction of *Juniperus virginiana* throughout the natural range (**12**, p. 63) of the native host species. During the early period of the cedar-blight investigation the writer searched unsuccessfully for the nursery parasite on native trees in the East in the vicinity of the District of Columbia where the disease and fructifications of the causal organism could be found readily on transplant nursery stock used for ornamental purposes. Within recent years subsequent studies of diseased wildling material from the Central States, particularly from southern Wisconsin not far from Iowa, where the blight has been destructive in nurseries for many years (**15**) failed to reveal the parasite although other fungi were present.

In 1939 Davis and Latham (**1**) reported the presence of *Phomopsis juniperovora* on diseased red cedar wildlings growing in North Carolina, Virginia, and Tennessee. They did not find fructifications of the parasite on this type of stock but were able to demonstrate its presence by tissue isolations, which in turn produced the imperfect stage.

During May of the current year the writer was afforded an opportunity to make a study of cedar blight in the Prairie States. In company with Dr. Wright, a brief but intensive search for the

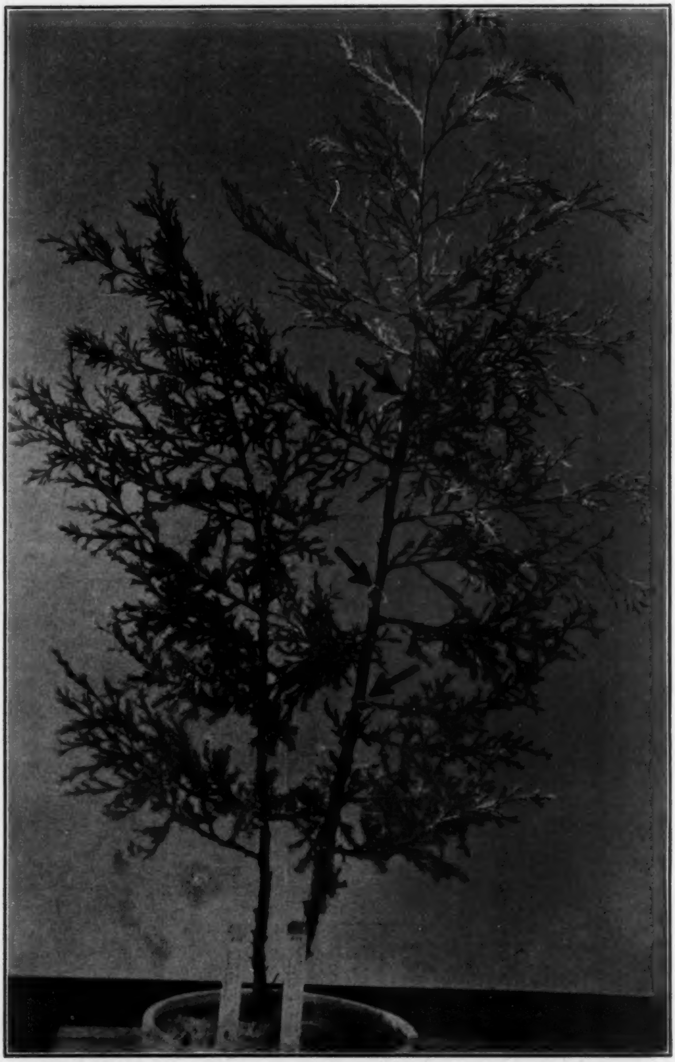

FIG. 2. The forked eastern red cedar wildling was tested on the right with *Phomopsis juniperovora* and similarly on the left with *P. occulta*. Only the tests with the cedar-blight pathogen succeeded. The infected incisions and check below are indicated by arrows. (Photographed by H. G. Eno, March 1942.)

parasite was made on cedars growing in native stands in regions where Federal nurseries troubled with the disease are located at Fremont, Nebraska and Manhattan, Kansas. In the time available for the study, *Phomopsis juniperovora* was discovered only in the Nebraska area, 7 miles from Fremont where cedar blight has been destructive in commercial and Federal nurseries during epidemic years at least since 1915 (**6**, p. 52). Here the nursery parasite was found on suppressed cedars that were growing in a damp situation in the Platte River bottom beneath a dense closed canopy of mixed hardwoods.

Fruiting bodies of *Phomopsis juniperovora,* the identity of which was proven by cultural tests, are reported on wildling red cedar for the first time. Not a large amount of the parasite was present and that which was found was located with difficulty on weathered, dead branchlet tips of old, suppressed cedars along with other microfungi. In the laboratory these pycnidia under moist conditions produced spore tendrils.

Dr. Wright and the writer were unable to find the pathogen in this same area, which is a source of seed for nursery propagation, on vigorous cedars growing in the open, or on trees or reproduction of all age classes including seedlings growing beneath cottonwoods. Although *Phomopsis juniperovora* was not found on the seed trees in the open, a conspicuous dying of twigs and small branches not caused by *Phomopsis* was very common.

In contrast with the meager amount of fruiting of *Phomopsis juniperovora* on wildlings in the Nebraska area just described, the collectors found abundant fruiting of the parasite on blighted branchlets of nursery cedars used as hedge plants in a Manhattan, Kansas nursery. Isolates from this nursery material were identical culturally with those taken from wildling specimens.

Although it is known that cedar blight has been disseminated on infected nursery stock, further information on the fruiting of the pathogen on wildling cedars is highly desirable in order that our knowledge of its natural distribution may be increased. Data should also be obtained on the problem of whether cedar blight is seed borne. Moreover our knowledge of red cedars highly resistant to the blight also should be increased. The solution of these problems has practical value in the control of the disease, particu-

larly in regions where *Phomopsis juniperovora* has not been reported and where there is a possibility of its nonoccurrence.

SUMMARY

Because of the close morphological similarity between the saprophyte, *Phomopsis occulta* (Trav.) Sacc., and the destructive nursery parasite, *P. juniperovora* Hahn, on eastern red cedar (*Juniperus virginiana* L.), the distribution of the latter as a possible endemic pathogen has been confused, particularly in cases where diagnoses of *Phomopsis* on wildling cedars are concerned.

Comparable wound inoculation tests carried on in an unheated greenhouse with monospore cultures of *Phomopsis occulta* and *P. juniperovora,* the latter serving as a "check" to establish the suitability of the environmental conditions, demonstrated that *P. occulta* was unable to attack any of the 14 wildling eastern red cedar saplings used for the tests. The *P. occulta* inocula were obtained from both wildling and nursery stock. Results of these tests corroborate similar ones obtained previously with *P. occulta* on Arizona cypress and Chinese arborvitae.

All of the 7 red cedars inoculated with *Phomopsis juniperovora* became infected. Three of the wildlings, which were bluish-green in color, were girdled and killed back toward the base of the plant, whereas two saplings, which were decidedly green in color, showed blight resistance. Infections on one of the latter, which was lighter and brighter in color, were confined to nongirdling cankers on the trunk, and the terminal and lateral branches did not show discoloration.

Reisolations of *P. juniperovora* on synthetic malt agar produced the yellow coloration and flaming orange crystals first reported in 1917. These characters are typical of the growth of the pathogen on a number of media. Neither the yellow color nor crystals appear in cultures of *P. occulta,* and they serve to differentiate distinctly the two *Phomopsis* species, the spore size ranges of which overlap.

In a discussion of the two related *Phomopsis* species it is pointed out that the results obtained by the writer in earlier studies demonstrated that *Phomopsis occulta* could be distinguished morphologically from *P. juniperovora,* that *P. occulta* is distributed widely

on conifers both in western Europe and throughout North America on numerous host genera, and that *Diaporthae* on conifers, including the perfect stage, *Diaporthe conorum* (Desm.) Niessl, of *P. occulta,* although common tin Europe, are exceedingly rare in the Western Hemisphere.

Unpublished data are given on experiments conducted in Great Britain during 1926 to 1929, in which the perfect stage, *Diaporthe conorum,* was obtained culturally on twigs of English elm (*Ulmus procera* Salisb.) from monopycnidiospores of the fungus originally isolated from cultures of monoascospores of *D. conorum* collected on Douglas-fir. These results tend to support Wehmeyer's treatment of a large *Diaporthae* complex on both hard and soft woods belonging to the type species, *Diaporthe eres* Nit. on *Acer,* although admittedly, the homothallic *D. conorum* holds priority.

Phomopsis occulta is to be regarded as a secondary fungus on cedars following injury by other factors. Previous studies carried on by the writer showed that under artificial conditions the organism at most was weakly parasitic on the coast form of Douglas-fir.

The pathogen, *Phomopsis juniperovora,* under natural conditions parasitizes only genera of the Cupressaceae. Its *Diaporthe* stage is unknown. A revised host list is given, which includes all of the host species known to be attacked up to the present time. Early studies by the writer demonstrated that under artificial conditions saplings of Douglas-fir, Coast form, were highly susceptible to *P. juniperovora.* Although nursery stock of Douglas-fir is not known to be attacked naturally by the cedar-blight parasite, the potentialities of *P. juniperovora* on juvenile stock of this host merit consideration.

Phomopsis juniperovora is reported to occur in Europe as a nursery parasite on the continent. As in the case of *P. occulta,* its perfect stage (*Diaporthe*) may be present, and there is the possibility that it may be discovered eventually on some obscure European host.

Although we have considerable information on the distribution of the cedar-blight parasite in commercial and Federal nurseries in this country, there is not a great amount of data available concerning the occurrence of the organism on trees and reproduction of

eastern red cedar in native stands. A small amount of fruiting bodies of *Phomopsis juniperovora* collected by Dr. Wright and the writer on suppressed red cedar wildlings, was confirmed later by culture tests and is reported for the first time.

Additional information on the occurrence of *Phomopsis juniperovora* on wildlings is highly desirable from the standpoint of increasing our knowledge concerning the dissemination of cedar blight. In regions where cedar blight has not been reported and where there is a possibility of its nonoccurrence, this information should prove of practical value in disease control.

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NOTES AND BRIEF ARTICLES

Correction

In paging the article "Some species of *Papulaspora* associated with rots of *Gladiolus* bulbs" by H. H. Hotson in the July–August, 1942 number of Mycologia, the first line of the second paragraph was accidentally dropped out by the printers. This has been corrected in the reprints but, unfortunately, cannot be changed in the issues which have been sent out. Therefore, please supply in its proper place on page 391, the following line:

"The material used was derived chiefly from diseased *Gladiolus*"

A Little Known Fungus

During a recent vacation trip to Bar Harbor, Maine, among other things, a large *Hydnum* was collected in spruce woods which attracted the writer's attention. On returning home it was identified as *Hydnum boreale,* described by Howard J. Banker in 1902 (Bull. Torrey Club **29**: 253.) from material collected at Bar Harbor in 1901 by V. S. White, and the type specimen deposited in the Garden herbarium. So far as can be discovered no specimen of this species, at least under this name, is preserved in any other herbarium. Two large specimens were obtained and a number of smaller ones, apparently young plants. In fact, I have reason to believe that the species is quite common on Mt. Desert Island and, doubtless, in other localities as well. We believe the species is sufficiently unusual to deserve mention at this time.—Fred J. Seaver.

Mycetozoa: A New Combination

I propose to include in the genus *Perichaena* of the Mycetozoa the two following forms heretofore in the genus *Hemitrichia.*

Perichaena minor (G. Lister) Hagelstein, comb. nov. (*Hemitrichia minor* G. Lister, Jour. Bot. **49**: 62. 1911); and **Perichaena**

minor (G. Lister) Hagelstein, var. **pardina** (Minakata) Hagelstein, comb. nov. (*Hemitrichia minor* G. Lister, var. *pardina* Minakata; G. Lister, Trans. Brit. Myc. Soc. **5**: 82. 1914).

The forms were placed in the genus *Hemitrichia* by Miss Lister, apparently in the belief that the faint, narrow, diagonal lines appearing occasionally on the threads of the capillitium were thickened, spiral bands, as found in that genus. A critical examination with an apochromatic objective of N. A. 1.4 of the capillitium of a fine collection of the two forms from Long Island, New York, shows that these apparent lines are not thickened, spiral bands, but merely the appearance of the arrangement, here and there, of minute spines on the threads, similar to the oblique markings seen on the siliceous valves of many species of the Diatomaceae, which latter are composed of minute puncta. In all other respects the characters of the two forms are those of the genus *Perichaena*.—ROBERT HAGELSTEIN.

WYNNEA AMERICANA IN WESTERN PENNSYLVANIA

While collecting fungi, 3 miles N.E. of Emlenton, Venango County, Pennsylvania, in September 1942, the writer found a small clump of *Wynnea americana*. This region is over 100 miles north of Pennsylvania's southern border. According to previously published records, this seems to be the northernmost range for this species.

In 1936 and again in 1937, the writer collected specimens from two localities, about two miles apart, 4 miles N.E. of Harmony, Butler County, Pennsylvania. These localities are in approximately the same latitude as Mansfield, Ohio, where specimens had already been reported.

The Carnegie Museum Herbarium has several other collections of this species, chiefly from the southern mountainous section. O. E. Jennings collected specimens at Ohio Pyle, Fayette County, Pennsylvania in 1905. These were noted and described by D. R. Sumstine in the Journal of Mycology (**12**: 59. March, 1906). In 1906 and 1907 additional specimens were obtained from the Ohio Pyle region and also from New Florence in northeastern Westmoreland County, Pennsylvania. These were reported in the

Annals of Carnegie Museum (Vol. IV, Nos. III & IV, April, 1908). D. R. Sumstine collected specimens at Idlewild Park near Ligonier in 1910 and at Jones Mills in 1936, both localities in Westmoreland County. In 1922 specimens were found by O. E. Jennings near Rector, Westmoreland County, and in 1932 by C. M. Hepner near Trent, Somerset County, Pennsylvania.—L. K. HENRY.